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Exhibit 1

(54) Title: A P53-INDUCED PROTEIN WITH A DEATH DOMAIN THAT CAN PROMOTE APOPTOSIS

(57) Abstract: The invention relates to novel nucleic acid molecules encoding a p53 induced protein with a death domain that can promote apoptosis; the novel proteins encoded by the nucleic acid molecules; and, uses of the proteins and nucleic acid molecules, especially in the treatment of cancer and dysplasia, autoimmune disease neurodegenerative disorders, or stroke.

Title **A p53-Induced Protein with a Death Domain that can Promote Apoptosis**

This application claims priority from United States provisional patent application no. 60/152,359 filed September 7, 2000 and United States provisional patent application no. 60/211,086 filed June 13, 2000, both of which are herein incorporated by reference.

FIELD OF THE INVENTION

The invention relates to a novel p53 induced protein with a death domain (PIDD) that can promote apoptosis, to nucleic acid molecules encoding the protein, fragments of the protein and nucleic acid molecules and to methods and uses thereof. In another embodiment, the invention relates to modulators of PIDD expression and/or activity.

BACKGROUND OF THE INVENTION

Entry of cells into the cell cycle from a quiescent state and progression of cells around the cycle is precisely controlled. Cell growth and coordination of DNA synthesis with cell size increase and cytokinesis are monitored. p53 is an important check point regulator of this control system. p53 can hold cells in check, quiescent, and prevent cells from becoming cancerous. It can also induce cell death (apoptosis) if conditions are not optimal. Cells that lack p53 lose the ability to check cell cycle progression, which can lead to increased rates of DNA alteration, mutations, and translocations. p53 deficiency can speed up evolution of new oncogenes, making cells particularly dangerous.

The p53 tumor suppressor gene is the most commonly mutated gene in human cancer. The ability of p53 to mediate cell cycle arrest or apoptosis in response to stress including DNA damage is considered to be important for its tumor suppression function (1). One reason tumors are believed to be resistant to chemotherapy and radiotherapy is that they have lost the p53-dependent apoptosis pathway. One strategy to render these tumors sensitive to treatment is to introduce a functional p53 gene and reinstate p53 protein expression or otherwise reinstate the p53-dependent apoptosis pathway.

The ability of p53 to inhibit cell growth is due, at least in part, to its ability to bind to specific DNA sequences and activate the transcription of target genes (2-4). A number of p53 regulated genes have been identified. One p53-target gene, $p21^{WAF1}$, encodes an inhibitor of cyclin-dependent kinases required for cell cycle progression. Abundant evidence shows that p21 functions as a p53 target gene to arrest cell growth. p53 has been shown to regulate transcription of a number of genes involved in apoptosis including *bax*, *fas*, and *DR5*; however, no consensus has emerged regarding the importance of these genes in DNA damage and the p53-dependent apoptosis pathway.

Mutations in genes linked to p53 expression have been found in cancer cells,

Li-Fraumeni Syndrome, dysplasia and various diseases where there is a failure of apoptosis. For instance, mdm-2 a negative regulator of p53 is over expressed in sarcoma and other tumors. As a result, there has been considerable effort to identify p53-regulated genes since these may provide insight into the mechanism underlying p53-mediated growth arrest and apoptosis. Genes and proteins linked to p53 and its signalling pathway or mode of action may play a role in regulating the cell cycle, apoptosis and various disease states, such as cancer.

SUMMARY OF THE INVENTION

The present inventors have identified and characterized a novel p53 induced protein with a death domain that can promote apoptosis (hereinafter referred to as PIDD) and the nucleic acid sequence encoding therefor.

In one embodiment the PIDD is a mammalian PIDD, and preferably a murine or human PIDD. Although, the PIDD and encoding nucleic acid sequence of the invention can be isolated and characterized from any tissue, it is preferably isolated and characterized from fibroblasts, erythroleukemia cells, hematopoietic cells, or cells from the spleen, kidney, lung, muscle, brain, liver, heart or testis, more preferably from the spleen, kidney or lung and most preferably from hematopoietic cells, erythroleukemia cells or fibroblast cells.

Accordingly, the present invention provides an isolated nucleic acid molecule comprising a sequence encoding a PIDD, preferably a murine *Pidd* or human *PIDD* and fragments thereof.

In a preferred embodiment, an isolated nucleic acid molecule is provided having a nucleic acid sequence as shown in Figures 5 (SEQ ID NO: 13) or 10 (SEQ ID NO: 16). Most preferably, the purified and isolated nucleic acid molecule comprises: (a) a nucleic acid sequence as shown in Figures 5 (SEQ ID NO: 13), or 10 (SEQ ID NO: 16) wherein T can also be U; (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which are homologous to (a) or (b); or, (d) a fragment of (a) to (c) that is at least 15 bases, preferably 20 to 30 bases, and which will hybridize to (a) to (c) under stringent hybridization conditions.

In another embodiment the invention provides an isolated nucleic acid molecule comprising SEQ ID NOS: 14, 15, 17, 18, 19, 20, 21, 22, 23 or 24 where T can also be U; (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which are homologous to (a) and (b); (d) a nucleic acid molecule differing from any of the nucleic acid molecules of (a) to (c) in codon sequences due to the degeneracy of the genetic code; or (e) a fragment of (a) to (d) that is at least 15 bases, preferably 20 to 30 bases, and which will hybridize to (a) to (c) under stringent hybridization conditions.

The present invention also includes the PIDD protein itself. Accordingly, the invention provides a polypeptide having an amino acid sequence of a PIDD. Preferably, the invention provides a polypeptide having either the human (SEQ ID NO: 2) or mouse (SEQ ID NO: 1) amino acid sequence as shown in Figure 2a. The invention also comprises peptides comprising fragments of the amino acid sequence of Figure 2a. Preferably the fragments comprise amino acid residues 797-877 (SEQ ID NO: 8) of the mouse sequence or amino acids 792-872 (SEQ ID NO: 9) of the human sequence of Figure 2a. In another embodiment the fragments comprise amino acid residues 131-291 (SEQ ID NO: 10) of the mouse sequence of Figure 2a or amino acids 126-286 (SEQ ID NO: 11) of the human sequence of Figure 2a. In another embodiment the fragments preferably comprise at least 14 amino acid residues and are most preferably antigenic. In one embodiment the fragment comprises SEQ ID NOS: 3, 4, 5, 6 or 7. In another embodiment the invention provides peptides encoded by a nucleic acid sequence of Figures 5 (SEQ ID NO:13), or 10 (SEQ ID NO: 16) or fragments thereof or an antisense nucleic acid molecule to all or part of the nucleic acid molecule encoding PIDD.

In one embodiment, the invention also provides a nucleic acid molecule of the invention operationally linked to an expression control sequence in a suitable expression vector. In another embodiment, the expression vector comprising the nucleic acid molecule of the invention is capable of being activated to express the peptide which is encoded by the nucleic acid molecule and is capable of being transformed or transfected into a suitable host cell. Such transformed or transfected cells are also encompassed with the scope of this invention.

The invention also provides a method of preparing a PIDD protein of the invention utilizing a nucleic acid molecule of the invention. In one embodiment, a method for preparing a PIDD protein of the invention is provided comprising: transforming a host cell with a recombinant expression vector comprising a nucleic acid sequence of the invention; (b) selecting transformed host cells from untransformed host cells; (c) culturing a selected transformed host cell under conditions which allow expression of the protein; and (d) isolating the protein.

The invention also encompasses an antibody specific for one or more epitopes of a protein of the invention, such as a peptide specific antibody or a polyclonal antibody, and more preferably a monoclonal antibody. The invention also encompasses methods for preparing the antibodies. Preferably the epitopes are selected from the group consisting of SEQ ID NOS: 1 to 12.

The invention also includes a method for detecting a disease associated with PIDD expression in an animal. "A disease associated with PIDD expression" as used

herein means any disease which can be affected or characterized by the level of PIDD expression. This includes, without limitation, diseases affected by, high, normal, reduced or non-existent expression of PIDD or expression of mutated PIDD. A disease associated with PIDD expression includes diseases associated with cell cycle regulation, particularly cell growth and apoptosis, for instance cancer, dysplasia, autoimmune disease, and Li-Fraumeni Syndrome. The method comprises assaying for the PIDD from a sample, such as a blood sample, a biopsy, or other cellular or tissue sample, from an animal susceptible of having such a disease. In one embodiment, the method comprises contacting the sample with an antibody of the invention which binds PIDD, and measuring the amount of antibody bound to PIDD in the sample, or unreacted antibody. In another embodiment, the method involves detecting the presence of a nucleic acid molecule having a sequence encoding a PIDD, comprising contacting the sample with a nucleotide probe which hybridizes with the nucleic acid molecule, preferably mRNA or cDNA to form a hybridization product under conditions which permit the formation of the hybridization product, and assaying for the hybridization product.

The invention further includes a kit for detecting a disease associated with PIDD expression in a sample comprising an antibody of the invention, preferably a monoclonal antibody. Preferably directions for its use is also provided. The kit may also contain reagents which are required for binding of the antibody to a PIDD protein in the sample.

The invention also provides a kit for detecting the presence of a nucleic acid molecule having a sequence encoding a polypeptide related to or analogous to a polypeptide of the invention, comprising a nucleotide probe which hybridizes with the nucleic acid molecule, reagents required for hybridization of the nucleotide probe with the nucleic acid molecule, and directions for its use.

The invention further provides a method of treating or preventing a disease associated with PIDD expression comprising administering an effective amount of an agent that activates, simulates or inhibits PIDD expression, as the situation requires, to an animal in need thereof. In a preferred embodiment, PIDD, a therapeutically active fragment thereof, or an agent which activates or simulates PIDD expression is administered to the animal in need thereof to treat cancer, dysplasia or Li-Fraumeni Syndrome. In another embodiment the disease is associated with over expression of PIDD or too much apoptosis, and the method of treatment comprises adminstration of an effective amount of an agent that inhibits PIDD expression such as an antibody to PIDD, a mutation thereof, or an antisense nucleic acid molecule to all or part of the *PIDD* gene.

In another embodiment, the invention further provides a method for

identifying modulators of *PIDD* expression or PIDD activity.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF DRAWINGS

The invention will now be described in relation to the drawings:

Figure 1 a, b, c, d and e are Northern blots illustrating the activation of *Pidd* gene expression by p53. In **Figure 1a,** RNA was isolated from parental DP16.1 cells (p53ts -) or DP16. 1/p53ts cells (p53ts +) after incubation at 32°C for the times indicated. 10 ug of RNA was run in each lane and hybridized to *Pidd* and $p21^{WAF-1}$(*Cdkn1a*) cDNA probes. Ethidium bromide staining of the RNA samples is presented as a loading control. In **Figure 1b,** PolyA+ RNA from multiple mouse tissues (Clontech) was hybridized with a *Pidd* cDNA probe. The mobility of RNA molecular weight markers is indicated on the left. As a control, the blot was stripped and reprobed with actin to ensure equal loading of RNA in each lane and to ensure that it was not degraded (lower panel). In **Figure 1c,** RNA was isolated from wild-type (p53 +/+) and p53 -/- early passage mouse embryonic fibroblasts (MEFs) before and after γ-irradiation with a dose of 6 Gy at the times indicated. The blot was probed with *Pidd* cDNA or $p21^{WAF-1}$ cDNA as indicated. In **Figure 1d,** RNA was prepared from individual clones of wild-type or p53 -/- MEFs transformed with HPV-16 E7 and H-rasV12. The blot was probed with *Pidd* cDNA. In **Figure 1e** RNA was prepared from human K562 and OCI/AML-4 cells before and 8 h after γ-irradiation with a dose of 6 Gy as indicated. RNA was prepared from MCF-7/p53ts expressing cells after incubation at 37 °C or 8 h after incubation at 32 °C. The blot was probed with human *PIDD* cDNA, stripped and reprobed with *GAPD* cDNA.

Figure 2 illustrates the amino acid sequence characterization of mouse and human PIDD and sequence comparison. **Figure 2a,** is the amino acid sequence of mouse (GenBank Accession No. AF274973, SEQ ID NO: 1) and human (GenBank Accession No. AF274972, SEQ ID NO:2) PIDD. Numbers on the right indicate the amino acid residue position. Identical residues are indicated by a dot and gaps indicated by a dash. The N-terminal 7 tandem leucine-rich repeats (LRRs) are printed in bold (SEQ ID NO: 10 for mouse and SEQ ID NO: 11 for human); the C-terminal death domain is placed inside the box (SEQ ID NO: 8 for mouse and SEQ ID NO: 9 for human). **Figure 2b,** illustrates the murine amino acid sequence alignment of the 7 tandem LRRs and the derived consensus

sequence; l represents L, I, V or A, and ". " represents any amino acid. **Figure 2c,** illustrates the amino acid sequence alignment of the predicted death domain of mouse and human PIDD with the death domains of other proteins: human RIP, aa 598-668 (21); human FADD, aa 101-180 (22, 23); human RAIDD, aa 123-199 (24, 25) ; and human DAPK, aa 1317-1396 (26). Residues identical in four or more proteins are shaded in black and those conserved in four or more proteins are shaded in grey. Homology shading was done with GeneDoc.

Figure 3 The p53-consensus binding sequence in *Pidd* is responsive to p53. In **Figure 3a,** the p53 binding sites in the 5'UTR of mouse *Pidd* (SEQ ID NO: 18) and human *PIDD* (SEQ ID NO: 19) are shown. The numbering is relative to the first nucleotide of the proposed ATG initiator methionine. Both sequences matched the published consensus sequence for a p53 DNA binding site (RRRCWWGYYYN{0,13}RRRCWWGYYY)(2), in which R is a purine, Y is a pyrimidine and W represents either an A or T residue (20 out of 20 matches for mouse *Pidd* and 19 out of 20 matches for human *PIDD*). In **Figure 3b,** electrophoretic mobility shift assays were performed with purified truncated p53 (residues 82-360). Binding reactions were performed with a ^{32}P-labelled double-stranded oligonucleotide containing the p53 consensus binding sequence in mouse *Pidd* or with an unrelated oligonucleotide (C) having the same nucleotide composition as the *Pidd* element. Unlabelled cold competitor (double-stranded *Pidd* or C oligonucleotide) was added at 10-fold, 50-fold and 100-fold molar excess over the labelled *Pidd* oligonucleotide. In **Figure 3c,** histogram representing the ability of wild-type p53 or the p53V143A mutant to transactivate luciferase reporters bearing the p53 binding sequence from *p21^{WAF1}* or mouse *Pidd*. SAOS2 cells were transfected with 5 µg luciferase reporter plasmids containing a minimal promoter consisting of a TATA box downstream of the p53 responsive element from the *p21^{WAF1}* promoter (p21-Luc) or the p53-binding consensus sequence present in mouse *Pidd* (Pidd-Luc) or a scrambled *Pidd* sequence (C-Luc), together with 5 µg pCDNA3 (empty vector control) or p53 expression vectors as indicated. Luciferase activity was measured 2 days post transfection. Transfections were performed in triplicate and the error bars indicate 1 standard error of the mean.

Figure 4 a and b are bar graphs illustrating that PIDD suppresses tumor cell growth and induces apoptosis. In **Figure 4a,** SAOS2 and K562 cells were co-transfected with pCDNA3 (vector control), pCDNA3-p53wt or pCDNA3-Pidd together with a plasmid expressing hygromycin resistance. Hygromycin-resistant colonies (>50 cells per colony) were enumerated 14 days after the start of selection. Bars represents the mean number of colonies obtained in three independent transfection experiments. Error bars indicate 1 standard error of the mean. In **Figure 4b,** K562 cells were co-transfected with pCDNA3, pCDNA3-p53wt or pCDNA3-Pidd together with pEGFP. Cells were stained with annexin V-PE and 7-AAD

and analyzed by flow cytometry. 5000 GFP-positive and 7-AAD negative cells were collected from each of three independent transfections. Data shown (mean ± s.e.m.) are the percentage of apoptotic cells staining with annexin V among the total number of cells collected.

Figure 5 shows the nucleic acid sequence of the murine p53 induced cDNA *(Pidd)* of the invention: The start and stop codons are indicated.(GenBank Accession No. AF274973, SEQ ID NO: 13)

Figure 6 shows a partial 1943 nucleic acid sequence of the human p53 induced gene of the invention indicating alignment with sequence of Figure 10 (SEQ ID NO:16). (It includes SEQ ID NO: 20 or SEQ ID NOS: 22 and 23 with a 175 base region between them).

Figure 7 is a table which shows the cDNA sequence percent homology between mouse Pidd (mPidd) death domain and human PIDD (hPIDD) and other death domains from other proteins: human RIP; human FADD; human RAIDD; and human DAPK.

Figure 8 shows the alignment of the nucleic acid sequence of the mouse *Pidd* (m*Pidd*) with that of the human (h*PIDD*).

Figure 9 shows the results of an apoptosis assay in which murine DP16.1/p53ts cells were exposed to *Pidd* antisense (AS), sense (S) or mismatched (MM) oligonucleotides. Three different antisense oligonucleotides were used: ODN-M76 (SEQ ID NO:25), ODN-M603 (SEQ ID NO: 26) and ODN-M837 (SEQ ID NO: 27). Cells were incubated in the presence of oligonucleotides at 32 °C for 16 h prior to assessment of apoptosis by Annexin V staining and flow cytometry. Human OCI/AML-4 cells were exposed to 6 Gy γ radiation in the presence or absence of *PIDD* AS, S or MM oligonucleotides (ODN-H144, SEQ ID NO: 28) and apoptosis was measured 16 h after irradiation by Annexin V staining and flow cytometry. Oligonucleotides were introduced into cells using streptolysin O reversible permeabilization with the exception of ODN-M76 which was introduced by association with a cationic lipid, lipofectin. Approximately 50% of the cells were positive for uptake of fluorescein isothiocyanate-labelled oligonucleotides by ultraviolet fluorescence microscopy and flow cytometry. The data presented have not been normalized to account for the incomplete uptake of the oligonucleotides.

Figure 10, is the full length nucleic acid sequence of the human p53 induced cDNA of the invention (h*PIDD)*, showing start and stop codons.(GenBank Accession No. AF274972, SEQ ID NO: 16)

Figure 11 is a Western blot of p53 induced Pidd expression. PIDD was first isolated from the sample by immunoprecipitation using the Pidd polyclonal antibody of the invention and then loaded on the gel. The Western blot is then probed with a labelled

polyclonal antibody of the invention and detected by enhanced chemoluminescence.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention is directed to novel p53 induced protein with a dealth domain, nucleic acid molecules encoding the protein and to antisense nucleic acid molecules thereto. The invention is further directed to fragments of the nucleic acid molecules and proteins of the invention; antibodies to the protein of the invention; and uses of the protein, nucleic acid molecules, and antibodies of the invention. Although, the examples in the present invention isolates and characterizes the PIDD protein and encoding cDNA from murine and human cells, a person skilled in the art would appreciate that the gene encoding for PIDD is present in the genome of many other animals.

"PIDD" as used herein refers generally to a p53 induced protein with a death domain and more particularly refers to human or both human or murine p53 induced protein with a death domain or fragment thereof as the context permits. "*PIDD*" as used herein refers to nucleic acid molecule encoding PIDD as defined above. "Pidd" as used herein refers to the murine p53 induced protein with a death domain or fragments thereof as the context permits. *"Pidd"* as used herein refers to the nucleic acid molecule encoding Pidd as defined above.

"Animal" as used herein is any animal of the animal kingdom which comprises a nucleic acid molecule encoding a PIDD and is thus potentially susceptible to disease associated with PIDD expression. Preferably, the animials are dogs, cats, mice, horses and humans. The *PIDD* coding sequence could be identified in any tissue of said animals, for instance, by using nucleotide probes derived from the sequences disclosed herein. *PIDD* expression is preferably found in cells such as hematopoietic cells, erythroleukemia cells, fibroblasts, and in tissues such as the spleen, kidney and lung.

The following standard abbreviations for the amino acid residues are used throughout the specification: A, Ala - alanine; C, Cys - cysteine; D, Asp- aspartic acid; E, Glu - glutamic acid; F, Phe - phenylalanine; G, Gly - glycine; H, His - histidine; I, Ile - isoleucine; K, Lys - lysine; L, Leu - leucine; M, Met - methionine; N, Asn - asparagine; P, Pro - proline; Q, Gln - glutamine; R, Arg - arginine; S, Ser - serine; T, Thr - threonine; V, Val - valine; W, Trp- tryptophan; Y, Tyr - tyrosine; and p.Y., P.Tyr - phosphotyrosine.

I. Nucleic Acid Molecules of the Invention

The present invention provides an isolated nucleic acid molecule comprising a sequence encoding a p53 induced protein with a death domain, PIDD.

The term "isolated" refers to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors, or other chemicals when chemically synthesized. The term "nucleic acid" is

intended to include DNA and RNA and can be either double stranded or single stranded.

In an embodiment of the invention, an isolated nucleic acid molecule is provided having a sequence which encodes a PIDD having an amino acid sequence as shown in Figure 2a (SEQ ID NO: 1 or 2).

Preferably, the isolated nucleic acid molecule comprises

(a) a nucleic acid sequence as shown in Figures 5, or 10 (SEQ ID NOS: 13 or 16) wherein T can also be U;

(b) nucleic acid sequences complementary to (a);

(c) nucleic acid sequences which are homologous to (a) or (b);

(d) a nucleic acid molecule differing from any of the nucleic acids of (a) to (c) in codon sequences due to the degeneracy of the genetic code; or

(e) a fragment of (a) to (d) that is at least 15 bases, preferably 20 to 30 bases, and which will hybridize to (a) to (d) under stringent hybridization conditions.

In another embodiment the invention provides a nucleic acid molecule comprising the coding region of murine or human PIDD (SEQ ID NOS: 14 and 17 respectively). In another embodiment the invention provides a nucleic acid molecule comprising the p53 binding region of murine or human *PIDD* (SEQ ID NOS: 18 and 19, respectively). In yet another embodiment, the invention provides a nucleic acid molecule comprising human PIDD sequence fragments, SEQ ID NOS: 20, 21, 22, 23, and 24.

In all of the sequences referred to above, T can also be U. The invention further encompasses, nucleic acid molecules which are complementary in sequence to nucleic acid molecules of the invention, fragments of the nucleic acid molecules of the invention, preferably at least 15 bases, and more preferably of at least 20 to 30 bases, and which will hybridize to the nucleic acid molecules of the invention under stringent hybridization conditions. The invention further encompasses nucleic acid molecules which differ from any of the nucleic acid molecules of the invention in codon sequences due to the degeneracy of the genetic code.

The invention also encompasses nucleic acid seqeunces or molecules which are analogs of the nucleic acid sequences and molecules described herein. The term "a nucleic acid sequence which is an analog" means a nucleic acid sequence which has been modified as compared to the sequences described herein, such as seqeunces of (a), (b), (c), (d), or (e), above wherein the modification does not alter the utility of the sequenceas described herein. The modified sequence or analog may have improved properties over the sequence shown in (a), (b),(c), (d) or (e). One example of a modification to prepare an analog is to replace one of the naturally occurring bases (i.e. adenine, guanine, cytosine or thymidine) of the sequence shown in Figure 5, Figure6 or Figure 10, or SEQ ID NOS. 13 - 28 with a

modified base such as such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8 amino guanine, 8-thiol guanine, 8-thiolalkyl guanines, 8-hydroxyl guanine and other 8-substituted guanines, other aza and deaza uracils, thymidines, cytosines, adenines, or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

Another example of a modification is to include modified phosphorous or oxygen heteroatoms in the phosphate backbone, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages in the nucleic acid molecule shown in Figure 5, 6, or 10 or SEQ ID NOS. 13 - 28. For example, the nucleic acid sequences may contain phosphorothioates, phosphotriesters, methyl phosphonates, and phosphorodithioates.

A further example of an analog of a nucleic acid molecule of the invention is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in the DNA (or RNA), is replaced with a polyamide backbone which is similar to that found in peptides (P.E. Nielsen, et al Science 1991, 254, 1497). PNA analogs have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. PNAs also bind stronger to a complimentary DNA sequence due to the lack of charge repulsion between the PNA strand and the DNA strand. Other nucleic acid analogs may contain nucleotides containing polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may have morpholino backbone structures (U.S. Pat. No. 5,034,506). The analogs may also contain groups such as reporter groups, a group for improving the pharmacokinetic or pharmacodynamic properties of nucleic acid sequence.

It will be appreciated that the invention includes nucleic acid molecules encoding truncations of proteins of the invention, and analogs and homologs of proteins of the invention and truncations thereof, as described below. It will further be appreciated that variant forms of nucleic acid molecules of the invention which arise by alternative splicing of an mRNA corresponding to a cDNA of the invention are encompassed by the invention. The invention further includes biologically active fragments of the nucleic acid molecules of the invention. Such fragments would include, but is not necessarily limited to any nucleic acid molecules which are beneficial in the modulation or simulation of PIDD activity or *Pidd* expression, or in the identification or production of such agents.

Further, it will be appreciated that the invention includes nucleic acid molecules comprising nucleic acid sequences having substantial sequence homology with the

nucleic acid sequences as shown in Figures 5, 6 or 10 and fragments thereof. The term "sequences having substantial sequence homology" means those nucleic acid sequences which have slight or inconsequential sequence variations from these sequences, i.e., the sequences function in substantially the same manner to produce functionally equivalent proteins. The variations may be attributable to local mutations or structural modifications.

Nucleic acid sequences having substantial homology include nucleic acid sequences having at least 85%, preferably 90-95% identity with the nucleic acid sequence as shown in Figures 5, 6 or 10. Nucleic acid sequences having at least a 50% homology with the sequence shown in Figures 5, 6, or 10 are also encompassed within the scope of the present invention.

Another aspect of the invention provides a nucleic acid molecule, and fragments thereof having at least 15 bases, which hybridize to nucleic acid molecules of the invention under hybridization conditions, preferably stringent hybridization conditions. Appropriate stringency conditions which promote DNA hybridization are known to those skilled in the art, or may be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the following may be employed: 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C. The stringency may be selected based on the conditions used in the wash step. For example, the salt concentration in the wash step can be selected from a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, such as at about 65°C.

Isolated nucleic acid molecules having sequences which differ from the nucleic acid sequence shown in Figures 5, 6, or 10 due to degeneracy in the genetic code are also within the scope of the invention. Such nucleic acids encode functionally equivalent proteins but differ in sequence from the above mentioned sequences due to degeneracy in the genetic code.

An isolated nucleic acid molecule of the invention which comprises DNA can be isolated by preparing a labelled nucleic acid probe based on all or part of the nucleic acid sequences as shown in Figures 5, 6, or 10 and using this labelled nucleic acid probe to screen an appropriate DNA library (e.g. a cDNA or genomic DNA library). For example, a genomic library isolated can be used to isolate a DNA encoding a novel protein of the invention by screening the library with the labelled probe using standard techniques. Nucleic acids isolated by screening of a cDNA or genomic DNA library can be sequenced by standard techniques.

An isolated nucleic acid molecule of the invention which is DNA can also be isolated by selectively amplifying a nucleic acid encoding a novel protein of the invention

using the polymerase chain reaction (PCR) methods and cDNA or genomic DNA. It is possible to design synthetic oligonucleotide primers from the nucleic acid sequence as shown in Figures 5, 6, or 10, for use in PCR. A nucleic acid can be amplified from cDNA or genomic DNA using these oligonucleotide primers and standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. It will be appreciated that cDNA may be prepared from mRNA, by isolating total cellular mRNA by a variety of techniques, for example, by using the guanidinium-thiocyanate extraction procedure of Chirgwin et al., Biochemistry, 18, 5294-5299 (1979). cDNA is then synthesized from the mRNA using reverse transcriptase (for example, Moloney MLV reverse transcriptase available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase available from Seikagaku America, Inc., St. Petersburg, FL).

An isolated nucleic acid molecule of the invention which is RNA can be isolated by cloning a cDNA encoding a novel protein of the invention into an appropriate vector which allows for transcription of the cDNA to produce an RNA molecule which encodes a protein of the invention. For example, a cDNA can be cloned downstream of a bacteriophage promoter, (e.g., a T7 promoter) in a vector, cDNA can be transcribed *in vitro* with T7 polymerase, and the resultant RNA can be isolated by standard techniques.

A nucleic acid molecule of the invention may also be chemically synthesized using standard techniques. Various methods of chemically synthesizing polydeoxynucleotides are known, including solid-phase synthesis which, like peptide synthesis, has been fully automated in commercially available DNA synthesizers (See e.g., Itakura et al. U.S. Patent No. 4,598,049; Caruthers et al. U.S. Patent No. 4,458,066; and Itakura U.S. Patent Nos. 4,401,796 and 4,373,071).

Determination of whether a particular nucleic acid molecule encodes a novel protein of the invention may be accomplished by expressing the cDNA in an appropriate host cell by standard techniques, and testing the activity of the protein using the methods as described herein. A cDNA having the activity of a novel protein of the invention so isolated can be sequenced by standard techniques, such as dideoxynucleotide chain termination or Maxam-Gilbert chemical sequencing or by automated DNA sequencing, to determine the nucleic acid sequence and the predicted amino acid sequence of the encoded protein.

The initiation codon and untranslated sequences of nucleic acid molecules of the invention may be determined using currently available computer software designed for the purpose, such as PC/Gene (IntelliGenetics Inc., Calif.). Regulatory elements can be identified using conventional techniques. The function of the elements can be confirmed by using these elements to express a reporter gene which is operatively linked to the elements.

These constructs may be introduced into cultured cells using standard procedures. In addition to identifying regulatory elements in DNA, such constructs may also be used to identify proteins interacting with the elements, using techniques known in the art.

The sequence of a nucleic acid molecule of the invention may be inverted relative to its normal presentation for transcription to produce an antisense nucleic acid molecule. The term "antisense" nucleic acid molecule is a nucleotide sequence that is complementary to its target. Preferably, an antisense sequence is constructed by inverting a region preceding or targeting the initiation codon or an unconserved region. In another embodiment the antisense sequence targets all or part of the mRNA or cDNA of *PIDD*. In particular, the nucleic acid sequences contained in the nucleic acid molecules of the invention or a fragment thereof, preferably a nucleic acid sequence shown in Figures 5, 6, or 10 may be inverted relative to its normal presentation for transcription to produce antisense nucleic acid molecules. Preferably, the antisense nucleic acid molecule of the invention is selected from the group consisting of: ODN-M76 9 (SEQ ID NO: 25), ODN-M603 (SEQ ID NO: 26), ODN-M837 (SEQ ID NO: 27) and ODN-H144 (SEQ ID NO: 28). In one embodiment the antisense molecules can be used to inhibit PIDD expression and/or apoptosis.

The antisense nucleic acid molecules of the invention or a fragment thereof, may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed with mRNA or the native gene e.g. phosphorothioate derivatives and acridine substituted nucleotides. The antisense sequences may be produced biologically using an expression vector introduced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense sequences are produced under the control of a high efficiency regulatory region, the activity of which may be determined by the cell type into which the vector is introduced.

The invention also provides nucleic acids encoding fusion proteins comprising a novel protein of the invention and a selected protein, or a selectable marker protein (see below).

II. Novel Proteins of the Invention

The invention further contemplates an isolated p53 induced protein having a death domain. In an embodiment of the invention, an isolated protein is provided which has either the mouse or human amino acid sequence as shown in Figure 2a (SEQ ID NO: 1 or 2). The present invention also encompasses peptides encoded by the nucleic acid sequence of Figure 5 or 10 (SEQ ID NO: 13 or 16) and all embodiments therefor as described in reference to the peptides shown in Figure 2a described below.

Within the context of the present invention, a protein of the invention may

include various structural forms of the primary protein which retain biological activity. For example, a protein of the invention may be in the form of acidic or basic salts or in neutral form. In addition, individual amino acid residues may be modified by oxidation or reduction.

In addition to the full length amino acid sequences (Figure 2a), the proteins of the present invention may also include truncations of the proteins, and analogs, and homologs of the proteins and truncations thereof as described herein. Truncated proteins may comprise peptides of at least 10 and preferably at least fourteen amino acid residues.

In embodiment the invention provides a peptide fragment of SEQ ID NOS: 1 or 2. In another embodiment the invention provides a peptide having an amino acid sequence comprising the PIDD death domain sequence, SEQ ID NOS: 8 or 9. In another embodiment the invention provides peptide fragment comprising the PIDD leucine rich repeat sequence, SEQ ID NO: 10 or 11. In yet another embodiment the invention provides an antigenic fragment of the proteins of the invention, such as peptides having SEQ ID NOS: 3, 4, 5, 6, and/or 7, most preferably SEQ ID NOS: 4, 5 and/or 7. In a further embodiment, the invention provides a peptide comprising SEQ ID NO: 12.

Analogs of the proteins having the amino acid sequences shown in Figure 2a and/or truncations thereof as described herein, may include, but are not limited to an amino acid sequence containing one or more amino acid substitutions, insertions, and/or deletions. Amino acid substitutions may be of a conserved or non-conserved nature. Conserved amino acid substitutions involve replacing one or more amino acids of the proteins of the invention with amino acids of similar charge, size, and/or hydrophobicity characteristics. When only conserved substitutions are made the resulting analog should be functionally equivalent. Non-conserved substitutions involve replacing one or more amino acids of the amino acid sequence with one or more amino acids which possess dissimilar charge, size, and/or hydrophobicity characteristics.

One or more amino acid insertions may be introduced into the amino acid sequences shown in Figure 2a. Amino acid insertions may consist of single amino acid residues or sequential amino acids ranging from 2 to 15 amino acids in length. For example, amino acid insertions may be used to destroy target sequences so that the protein is no longer active. This procedure may be used *in vivo* to inhibit the activity of a protein of the invention.

Deletions may consist of the removal of one or more amino acids, or discrete portions from the amino acid sequence shown in Figures 2a. The deleted amino acids may or may not be contiguous. The lower limit length of the resulting analog with a deletion mutation is about 10 amino acids, preferably 100 amino acids.

Analogs of the proteins of the invention may be prepared by introducing mutations in the nucleotide sequence encoding the protein. Mutations in nucleotide sequences constructed for expression of analogs of a protein of the invention must preserve the reading frame of the coding sequences. Furthermore, the mutations will preferably not create complementary regions that could hybridize to produce secondary mRNA structures, such as loops or hairpins, which could adversely affect translation of the mRNA.

Mutations may be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site specific mutagenesis procedures may be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Deletion or truncation of a protein of the invention may also be constructed by utilizing convenient restriction endonuclease sites adjacent to the desired deletion. Subsequent to restriction, overhangs may be filled in, and the DNA religated. Exemplary methods of making the alterations set forth above are disclosed by Sambrook et al (Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1989).

Insertions, deletions or substitution mutations of PIDD can be used to generate dominant negative forms of PIDD that can act as transdominant repressors of PIDD activity.

The proteins of the invention also include homologs of the amino acid sequence shown in Figure 2a and/or truncations thereof as described herein. Such homologs are proteins whose amino acid sequences are encoded by nucleic acid sequences that hybridize under stringent hybridization conditions (see discussion of stringent hybridization conditions herein) with a probe used to obtain a protein of the invention. Homologs of a protein of the invention will have the same regions which are characteristic of the protein.

A homologous protein includes a protein with an amino acid sequence having at least 76%, preferably 80-90% identity with the amino acid sequence as shown in Figure 2a.

The invention also contemplates isoforms of the proteins of the invention. An isoform contains the same number and kinds of amino acids as a protein of the invention, but the isoform has a different molecular structure. The isoforms contemplated by the present invention are those having the same properties as a protein of the invention as described herein.

The present invention also includes a protein of the invention conjugated with a selected protein, or a selectable marker protein (see below) to produce fusion proteins. Additionally, immunogenic portions of a protein of the invention are within the scope of the invention.

The proteins of the invention (including truncations, analogs, etc.) may be prepared using recombinant DNA methods. These proteins may be purified and/or isolated to various degrees using techniques known in the art. Accordingly, nucleic acid molecules of the present invention having a sequence which encodes a protein of the invention may be incorporated according to procedures known in the art into an appropriate expression vector which ensures good expression of the protein. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), so long as the vector is compatible with the host cell used. The expression "vectors suitable for transformation of a host cell", means that the expression vectors contain a nucleic acid molecule of the invention and regulatory sequences, selected on the basis of the host cells to be used for expression, which are operatively linked to the nucleic acid molecule. "Operatively linked" is intended to mean that the nucleic acid is linked to regulatory sequences in a manner which allows expression of the nucleic acid.

The invention therefore contemplates a recombinant expression vector of the invention containing a nucleic acid molecule of the invention, or a fragment thereof, and the necessary regulatory sequences for the transcription and translation of the inserted protein-sequence. Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, or viral genes (For example, see the regulatory sequences described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Selection of appropriate regulatory sequences is dependent on the host cell chosen, and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector. It will also be appreciated that the necessary regulatory sequences may be supplied by the native protein and/or its flanking regions.

The invention further provides a recombinant expression vector comprising a DNA nucleic acid molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a

manner which allows for expression, by transcription of the DNA molecule, of an RNA molecule which is antisense to a nucleotide sequence comprising the nucleotides as shown in Figure 5 or 10 or fragments thereof. Regulatory sequences operatively linked to the antisense nucleic acid can be chosen which direct the continuous expression of the antisense RNA molecule.

The recombinant expression vectors of the invention may also contain a selectable marker gene which facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention. Examples of selectable marker genes are genes encoding a protein which confers resistance to certain drugs, such as G418 and hygromycin. Examples of other markers which can be used are: green fluorescent protein (GFP), β-galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. Transcription of the selectable marker gene is monitored by changes in the concentration of the selectable marker protein such as β-galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. If the selectable marker gene encodes a protein conferring antibiotic resistance such as neomycin resistance transformant cells can be selected with G418. Cells that have incorporated the selectable marker gene will survive, while the other cells die. This makes it possible to visualize and assay for expression of recombinant expression vectors of the invention and in particular to determine the effect of a mutation on expression and phenotype. It will be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

The recombinant expression vectors may also contain genes which encode a fusion moiety which provides increased expression of the recombinant protein; increased solubility of the recombinant protein; and aid in the purification of a target recombinant protein by acting as a ligand in affinity purification. For example, a proteolytic cleavage site may be added to the target recombinant protein to allow separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein.

Recombinant expression vectors can be introduced into host cells to produce a transformed host cell. The term "transformed host cell" is intended to include prokaryotic and eukaryotic cells which have been transformed or transfected with a recombinant expression vector of the invention. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (e.g. a vector) into a cell by one of many possible techniques known in the art. Prokaryotic cells can be transformed with nucleic acid by, for example, electroporation or calcium-chloride mediated transformation. Nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofectin, electroporation or

microinjection. Suitable methods for transforming and transfecting host cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other such laboratory textbooks.

Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the proteins of the invention may be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus), yeast cells or mammalian cells. Other suitable host cells can be found in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1991).

The proteins of the invention may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, J. Am. Chem. Assoc. 85:2149-2154) or synthesis in homogenous solution (Houbenweyl, 1987, Methods of Organic Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart).

III. Applications

1. Diagnostic Applications

The above nucleic acid and peptide molecules of the invention can be used to diagnose a disease affected by PIDD expression, such as a disease associated with the regulation of the cell cycle or apoptosis, such as cancer, dysplasia, various autoimmune diseases, and Li-Fraumeni Syndrome.

(i) Nucleic Acids

The above described nucleic acid molecules of the invention, allow those skilled in the art to construct nucleotide probes for use in the detection of nucleotide sequences homologous to *PIDD* or a fragment thereof in a sample.

Accordingly, the present invention also relates to a method of detecting the presence of nucleic acid molecules encoding a PIDD in a sample comprising contacting the sample under hybridization conditions with one or more nucleotide probes which hybridize to the nucleic acid molecules and are labelled with a detectable marker, and, determining the degree of hybridization between the nucleic acid molecule in the sample and the nucleotide probe(s).

A nucleotide probe may be labelled with a detectable marker such as a radioactive label which provides for an adequate signal and has sufficient half life such as ^{32}P, ^{3}H, ^{14}C or the like. Other detectable markers which may be used include antigens that are recognized by a specific labelled antibody, fluorescent compounds, enzymes, antibodies specific for a labelled antigen, and chemiluminescent compounds. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to the nucleotide to be detected and the amount of nucleotide available for hybridization.

Hybridization conditions which may be used in methods of the invention are known in the art and are described for example in Sambrook J, Fritch EF, Maniatis T. In: Molecular Cloning, A Laboratory Manual,1989. (Nolan C, Ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. The hybridization product may be assayed using techniques known in the art. The nucleotide probe may be labelled with a detectable marker as described herein and the hybridization product may be assayed by detecting the detectable marker or the detectable change produced by the detectable marker.

A nucleic acid molecule of the invention also permits the identification and isolation, or synthesis of nucleotide sequences which may be used as primers to amplify a nucleic acid molecule of the invention, for example, in a polymerase chain reaction (PCR) which is discussed in more detail below. The primers may be used to amplify the genomic DNA of other *PIDD* genes. The PCR amplified sequences can be examined to determine the relationship between the various *PIDD* genes.

The length and bases of primers for use in a PCR are selected so that they will hybridize to different strands of the desired sequence and at relative positions along the sequence such that an extension product synthesized from one primer when it is separated from its template can serve as a template for extension of the other primer into a nucleic acid of defined length. Primers which may be used in the invention are oligonucleotides, i.e., molecules containing two or more deoxyribonucleotides of the nucleic acid molecule of the invention which occur naturally as in a purified restriction endonuclease digest or are produced synthetically using techniques known in the art such as for example phosphotriester and phosphodiester methods (See Good *et al.* Nucl. Acid Res 4:2157, 1977) or automated techniques (See for example, Conolly, B.A. Nucleic Acids Res. 15:15(7): 3131, 1987). The primers are capable of acting as a point of initiation of synthesis when placed under conditions which permit the synthesis of a primer extension product which is complementary to a DNA sequence of the invention, i.e., in the presence of nucleotide substrates, an agent for polymerization such as DNA polymerase and at suitable temperature and pH. Preferably, the primers are sequences that do not form secondary structures by base pairing with other copies of the primer or sequences that form a hair pin configuration. The primer preferably contains between about 7 and 25 nucleotides.

The primers may be labelled with detectable markers which allow for detection of the amplified products. Suitable detectable markers are radioactive markers such as P-32, S-35, I-125, and H-3, luminescent markers such as chemiluminescent markers, preferably luminol, and fluorescent markers, preferably dansyl chloride, fluorcein-5-isothiocyanate, and 4-fluor-7-nitrobenz-2-axa-1,3 diazole, enzyme markers such as horseradish peroxidase, alkaline phosphatase, ß-galactosidase,

acetylcholinesterase, or biotin.

It will be appreciated that the primers may contain non-complementary sequences provided that a sufficient amount of the primer contains a sequence which is complementary to a nucleic acid molecule of the invention or oligonucleotide fragment thereof, which is to be amplified. Restriction site linkers may also be incorporated into the primers allowing for digestion of the amplified products with the appropriate restriction enzymes facilitating cloning and sequencing of the amplified product.

In an embodiment of the invention a method of determining the presence of a nucleic acid molecule having a sequence encoding a protein of the invention is provided comprising treating the sample with primers which are capable of amplifying the nucleic acid molecule or a predetermined oligonucleotide fragment thereof in a polymerase chain reaction to form amplified sequences, under conditions which permit the formation of amplified sequences and, assaying for amplified sequences.

Polymerase chain reaction as used herein refers to a process for amplifying a target nucleic acid sequence as generally described in Innis et al, Academic Press, 1990 in Mullis el al., U.S. Pat. No. 4,863,195 and Mullis, U.S. Patent No. 4,683,202. Conditions for amplifying a nucleic acid template are described in M.A. Innis and D.H. Gelfand, PCR Protocols, A Guide to Methods and Applications M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White eds, pp3-12, Academic Press 1989.

The amplified products can be isolated and distinguished based on their respective sizes using techniques known in the art. For example, after amplification, a DNA sample can be separated on an agarose gel and visualized, after staining with ethidium bromide, under ultra violet (uv) light. DNA may be amplified to a desired level and a further extension reaction may be performed to incorporate nucleotide derivatives having detectable markers such as radioactive labelled or biotin labelled nucleoside triphosphates. The primers may also be labelled with detectable markers as discussed above. The detectable markers may be analyzed by restriction enzyme digestion and electrophoretic separation or other techniques known in the art.

Conditions which may be employed in the methods of the invention using PCR are those which permit hybridization and amplification reactions to proceed in the presence of DNA in a sample and appropriate complementary hybridization primers. Conditions suitable for a polymerase chain reaction are generally known in the art. For example, see M.A. Innis and D.H. Gelfand, PCR Protocols, A guide to Methods and Applications M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White eds, pp3-12, Academic Press 1989. To amplify DNA template strands, preferably, the PCR utilizes polymerase obtained from the thermophilic bacterium *Thermus aquatics* (Taq polymerase, GeneAmp

Kit, Perkin Elmer Cetus) or other thermostable polymerase.

It will be appreciated that other techniques such as the Ligase Chain Reaction (LCR) and NASBA may be used to amplify a nucleic acid molecule of the invention (Barney in "PCR Methods and Applications", August 1991, Vol.1(1), page 5, and European Published Application No. 0320308, published June 14, 1989, and U.S. Serial NO. 5,130,238 to Malek).

(ii) Antibodies

A PIDD protein of the invention or antigenic portion thereof can be used to prepare antibodies specific for the protein. Antibodies can be prepared which bind a distinct epitope in an unconserved region of the protein. An unconserved region of the protein is one which does not have substantial sequence homology to other proteins. Alternatively, a region from a well-characterized domain can be used to prepare an antibody to a conserved region of a protein of the invention. Antibodies having specificity for a protein of the invention may also be raised from fusion proteins.

Conventional methods can be used to prepare the antibodies. For example, by using a peptide of a protein of the invention, polyclonal antisera or monoclonal antibodies can be made using standard methods. A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the peptide which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a peptide include conjugation to carriers or other techniques well known in the art. For example, the peptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay procedures can be used with the immunogen as antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera.

To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, (e.g., the hybridoma technique originally developed by Kohler and Milstein (Nature 256, 495-497 (1975)) as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., Immunol. Today 4, 72 (1983)); the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. Monoclonal Antibodies in Cancer Therapy (1985) Allen R. Bliss, Inc., pages 77-96); and screening of combinatorial antibody libraries (Huse et al., Science 246, 1275 (1989)). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the peptide and the monoclonal antibodies can be isolated.

Therefore, the invention also contemplates hybridoma cells secreting monoclonal antibodies with specificity for a protein of the invention.

The term "antibody" as used herein is intended to include fragments thereof which also specifically react with a protein of the invention, or peptide thereof. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above. For example, $F(ab')_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

Chimeric antibody derivatives, i.e., antibody molecules that combine a non-human animal variable region and a human constant region are also contemplated within the scope of the invention. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. Conventional methods may be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes a PIDD protein of the invention (See, for example, Morrison et al., Proc. Natl Acad. Sci. U.S.A. 81,6851 (1985); Takeda et al., Nature 314, 452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom patent GB 2177096B).

Monoclonal or chimeric antibodies specifically reactive with a protein of the invention as described herein can be further humanized by producing human constant region chimeras, in which parts of the variable regions, particularly the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. Such immunoglobulin molecules may be made by techniques known in the art (e.g., Teng et al., Proc. Natl. Acad. Sci. U.S.A., 80, 7308-7312 (1983); Kozbor et al., Immunology Today, 4, 7279 (1983); Olsson et al., Meth. Enzymol., 92, 3-16 (1982); and PCT Publication WO92/06193 or EP 0239400). Humanized antibodies can also be commercially produced (Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.)

Specific antibodies, or antibody fragments reactive against a protein of the invention may also be generated by screening expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with peptides produced from nucleic acid molecules of the present invention. For example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries (See for example Ward et al., Nature 341, 544-546: (1989); Huse et al., Science 246, 1275-1281 (1989); and McCafferty et al. Nature 348, 552-554 (1990)).

The antibodies may be labelled with a detectable marker including various

enzymes, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, ß-galactosidase, or acetylcholinesterase; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include S-35, Cu-64, Ga-67, Zr-89, Ru-97, Tc-99m, Rh-105, Pd-109, In-111, I-123, I-125, I-131, Re-186, Au-198, Au-199, Pb-203, At-211, Pb-212 and Bi-212. The antibodies may also be labelled or conjugated to one partner of a ligand binding pair. Representative examples include avidin-biotin and riboflavin-riboflavin binding protein. Methods for conjugating or labelling the antibodies discussed above with the representative labels set forth above may be readily accomplished using conventional techniques.

Antibodies reactive against PIDD proteins of the invention (e.g., enzyme conjugates or labelled derivatives) may be used to detect a protein of the invention in various samples, for example they may be used in any known immunoassays which rely on the binding interaction between an antigenic determinant of a protein of the invention and the antibodies. Examples of such assays are radioimmunoassays, western immunoblotting, enzyme immunoassays (e.g., ELISA), immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination, and histochemical tests. Thus, the antibodies may be used to identify or quantify the amount of a protein of the invention in a sample.

A sample may be tested for the presence or absence of a PIDD by contacting the sample with an antibody specific for an epitope of a PIDD protein which antibody is capable of being detected after it becomes bound to a PIDD protein in the sample, and assaying for antibody bound to a PIDD protein in the sample, or unreacted antibody.

In a method of the invention a predetermined amount of a sample or concentrated sample is mixed with antibody or labelled antibody. The amount of antibody used in the method is dependent upon the labelling agent chosen. The resulting protein bound to antibody or labelled antibody may be isolated by conventional isolation techniques, for example, salting out, chromatography, electrophoresis, gel filtration, fractionation, absorption, polyacrylamide gel electrophoresis, agglutination, or combinations thereof.

The sample or antibody may be insolubilized, for example, the sample or antibody can be reacted using known methods with a suitable carrier. Examples of suitable carriers are Sepharose or agarose beads. When an insolubilized sample or antibody is used protein bound to antibody or unreacted antibody is isolated by washing. For example, when the sample is blotted onto a nitrocellulose membrane, the antibody bound to a protein of the

invention is separated from the unreacted antibody by washing with a buffer, for example, phosphate buffered saline (PBS) with bovine serum albumin (BSA).

When labelled antibody is used, the presence of a PIDD can be determined by measuring the amount of labelled antibody bound to a protein of the invention in the sample or of the unreacted labelled antibody. The appropriate method of measuring the labelled material is dependent upon the labelling agent.

When unlabelled antibody is used in a method of the invention, the presence of a PIDD can be determined by measuring the amount of antibody bound to the PIDD using substances that interact specifically with the antibody to cause agglutination or precipitation. In particular, labelled antibody against an antibody specific for a protein of the invention, can be added to the reaction mixture. The antibody against an antibody specific for a protein of the invention can be prepared and labelled by conventional procedures known in the art which have been described herein. The antibody against an antibody specific for a protein of the invention may be a species specific anti-immunoglobulin antibody or monoclonal antibody, for example, goat anti-rabbit antibody may be used to detect rabbit antibody specific for a protein of the invention.

(iii) Kits

Reagents suitable for conducting the above described diagnostic methods of the invention may be packaged into convenient kits providing the necessary materials, packaged into suitable containers. Such kits may include all the reagents required to detect PIDD in a sample by means of the methods described herein, and optionally suitable supports useful in performing the methods of the invention.

In one embodiment of the invention the kit contains a nucleotide probe which hybridizes with a nucleic acid molecule of the invention, reagents required for hybridization of the nucleotide probe with the nucleic acid molecule, and directions for its use. In another embodiment of the invention the kit includes antibodies of the invention and reagents required for binding of the antibody to a PIDD protein in a sample. In still another embodiment of the invention, the kit includes primers which are capable of amplifying a nucleic acid molecule of the invention or a predetermined oligonucleotide fragment thereof, all the reagents required to produce the amplified nucleic acid molecule or predetermined fragment thereof in the polymerase chain reaction, and means for assaying the amplified sequences.

The methods and kits of the present invention have many practical applications. For example, the methods and kits of the present invention may be used to detect PIDD in any medical sample suspected of containing or lacking PIDD and used to diagnose diseases associated with PIDD expression such as cancer, dysplasia, certain

autoimmune diseases or Li-Fraumeni Syndrome. Samples which may be tested include bodily materials such as blood, urine, serum, tears, saliva, feces, tissues and the like.

Before testing a sample in accordance with the methods described herein, the sample may be concentrated using techniques known in the art, such as centrifugation and filtration. For hybridization and/or PCR-based methods described herein, nucleic acids may be extracted from cell extracts of the test sample using techniques known in the art.

2. Therapeutic Applications

Methods of Treatment/Pharmaceutical Compositions

PIDD may play a role in a number of diseases, such as those associated with regulation of the cell cycle or apoptosis. In particular PIDD may play a role in cancer or dysplasia by activating apoptosis. As such , the invention comprises methods for modulating or simulating PIDD activty or *PIDD* expression, preferably for treating or preventing a PIDD related condition. The invention further comprises uses of the modulating or simulating agents disclosed herein for the preparation of a medicament for treating or preventing a condition associated with PIDD expression or activity. In another embodiment the invention provides a use of the modulating or simulating agents for the treatment or prevention of a PIDD related condition.

Accordingly, the present invention provides a method of treating or preventing a disease associated with PIDD expression or activity comprising administering an agent that modulates or simulates PIDD expression or activity to an animal in need thereof, such as in an animal with cancer, dysplasia an autoimmune disease, or Li-Fraumeni Syndrome or certain viral infections.

In a preferred embodiment, preferably such agents stimulate or simulate PIDD activity. Examples of agents that activate or simulate PIDD activity would include without limitations, PIDD, the gene encoding for PIDD with suitable promoters, such promoters preferably being tissue specific promoters and therapeutically effective fragments of the nucleic acid and amino acid sequences of the invention.

Although, the inhibition of PIDD may reduce an animals ability to purge the body of cancerous cells there may be diseases or conditions in which inhibition of PIDD may be required, such as may be required to prevent tissue destruction caused by strokes or diseases such as certain autoimmune diseases or neurodegenerative diseases. Accordingly, the invention provides a method for treating or preventing a disease or condition associated with PIDD expression or activity by administering to a patient in need thereof an agent which inhibits or supresses PIDD expression or activity.

Examples of agents that inhibit PIDD include antisense nucleic acid

molecules, antibodies and transdominant inhibitors, as described herein.

Agents that inhibit, activate, or stimulate PIDD can be formulated into pharmaceutical compositions for adminstration to subjects in a biologically compatible form suitable for administration *in vivo*. As used herein "biologically compatible form suitable for administration *in vivo*" means a form of the substance to be administered in which therapeutic effects outweigh any toxic effects. The substances may be administered to animals in need thereof. Animals, as used herein refers to any animal susceptible to a disease associated with PIDD expression preferably dogs, cats, mice, horses and humans.

Administration of an "effective amount" of pharmaceutical compositions of the present invention is defined as an amount of the pharmaceutical composition, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance may vary according to factors such as disease state, age, sex, and weight of the recipient, and the ability of the substance to elicit a desired response in the recipient. Dosage regima may be adjusted to provide an optimum Therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

An active substance may be administered in a convenient manner such as by injection (subcutaneous, intravenous, topical, intratumoral etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active substance may be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound.

The compositions described herein can be prepared by known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

Recombinant nucleic acid molecules comprising a sense, an antisense sequence or oligonucleotide fragment thereof, may be directly introduced into cells or tissues *in vivo* using delivery vehicles known in the art such as retroviral vectors, adenoviral vectors and DNA virus vectors. They may also be introduced into cells *in vivo* using physical techniques

known in the art such as microinjection and electroporation or chemical methods such as coprecipitation and incorporation of DNA into liposomes. Recombinant molecules may also be delivered in the form of an aerosol or by lavage.

The utility of the substances, antibodies, sense and antisense nucleic acid molecules, and compositions of the invention may be confirmed in animal experimental model systems. Suitable animal model systems which can be used to determine Pidd activity may include, but is not limited to p53 or Pidd knock-out transgenic animals.

3. Other Applications

Screening for PIDD Modulating Compounds

In another embodiment, the invention provides a method for identifying a compound or molecule that modulates PIDD protein activity or gene expression. "Modulate" as used herein can include activation or increase of PIDD protein activity or gene expression or suppression of PIDD protein activity or gene expression The method includes incubating components comprising the compound and PIDD peptide or a recombinant cell expressing PIDD peptide, under conditions sufficient to allow the components to interact and determining the effect of the compound on PIDD activity or expression. The effect of the compound on PIDD activity can be measured by a number of assays and may include measurements before and after incubation in the presence of the compound. Compounds that affect PIDD activity or gene expression include peptides, chemical compounds and biologic agents. Assays include Northern blot analysis of *PIDD* mRNA (for gene expression), Western blot analysis (for protein level) and luciferase, apoptosis or growth suppression assays (for protein activity).

The above screening assays may be used for detecting the compounds or molecules that bind to the PIDD protein or peptide, in isolating molecules that bind to the PIDD gene, for measuring the amount of PIDD in a sample, either peptide or RNA (mRNA), for identifying molecules that may act as agonists or antagonists, and the like.

Incubating includes conditions which allow contact between the test compound and PIDD peptide or with a recombinant cell expressing PIDD peptide. Contacting includes in solution and in solid phase, or in a cell. The test compound may optionally be a combinatorial library for screening a plurality of compounds. Compounds identified in the method of the invention can be further evaluated, detected, cloned, sequenced and the like, either in solution or after binding to a solid support by any method usually applied to the detection of a specific DNA sequence such as PCT, oligomer restriction, allele-specific oligonucleotide probe analysis, and the like.

Screening for PIDD Related Disorders

Method for screening of PIDD protein activity and or gene expression as described above, can also be used to screen for PIDD related disorders. For instance, biological samples from patients with a particular conditions, such as leukemia, can be screened for PIDD protein activity and or gene expression. *PIDD* gene can also be sequenced from patients with a disorder to identify any mutations in the *PIDD* gene. Correlation between PIDD activity and/or gene expression/ and or any mutations and the disorder can be determined by a number of methods known in the art. For instance, PIDD activity and/or gene expression of a subject to be screened can be compared with that from "healthy" individuals. In one embodiment, the level of PIDD activity and/or gene expression can be compared with a cut off level for normal PIDD activity and/or gene expression. In one embodiment, the cutoff level can be determined by analysis of a database of levels from "healthy" individuals.

Transgenic Animals and Methods of Making Same

Nucleic acids which encode proteins having biological activity of PIDD can be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. Preferably, non-human transgenic animals are encompassed within the scope of this invention. A transgenic animal (e.g., a mouse) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, a human *PIDD* cDNA or mouse *Pidd cDNA*, comprising the nucleotide sequence shown in Figure 10, or an appropriate sequence thereof, can be used to clone a murine *Pidd* gene in accordance with established techniques and the genomic nucleic acid used to generate transgenic animals that contain cells which express Pidd protein. Methods for generating transgenic animals, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, 5,616, 491. In a preferred embodiment, plasmids containing recombinant molecules of the invention are microinjected into mouse embryos. In particular, the plasmids are microinjected into the male pronuclei of fertilized one-cell mouse eggs; the injected eggs are transferred to pseudo-pregnant foster females; and, the eggs in the foster females are allowed to develop to term. [Hogan, B. et al., (1986) A Laboratory Manual, Cold Spring Harbor, New York, Cold Spring Harbor Laboratory]. Alternatively, an embryonal stem cell line can be transfected with an expression vector containing nucleic acid encoding a protein having Pidd activity and cells

containing the nucleic acid can be used to form aggregation chimeras with embryos from a suitable recipient mouse strain. The chimeric embryos can then be implanted into a suitable pseudopregnant female mouse of the appropriate strain and the embryo brought to term. Progeny harbouring the transfected DNA in their germ cells can be used to breed uniformly transgenic mice.

Typically, particular cells would be targeted for Pidd transgene incorporation by use of tissue specific enhancers operatively linked to the Pidd-encoding gene. For example, promoters and/or enhancers which direct expression of a gene to which they are operatively linked preferentially in cardiac muscle cells can be used to create a transgenic animal which expresses a Pidd protein. Examples of suitable promoters and enhancers include those which regulate the expression of the genes for cardiac myosin and cardiac actin. Transgenic animals that include a copy of a *Pidd* transgene introduced into the germ line of the animal at an embryonic stage can also be used to examine the effect of increased Pidd expression in various tissues.

The pattern and extent of expression of a recombinant molecule of the invention in a transgenic mouse is facilitated by fusing a reporter gene to the recombinant molecule such that both genes are co-transcribed to form a polycistronic mRNA. The reporter gene can be introduced into the recombinant molecule using conventional methods such as those described in Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory press. Efficient expression of both cistrons of the polycistronic mRNA encoding the protein of the invention and the reporter protein can be achieved by inclusion of a known internal translational initiation sequence such as that present in poliovirus mRNA. The reporter gene should be under the control of the regulatory sequence of the recombinant molecule of the invention and the pattern and extent of expression of the gene encoding a protein of the invention can accordingly be determined by assaying for the phenotype of the reporter gene. Preferably the reporter gene codes for a phenotype not displayed by the host cell and the phenotype can be assayed quantitatively. Examples of suitable reporter genes include lacZ (β-galactosidase), neo (neomycin phosphotransferase), CAT (chloramphenicol acetyltransferase) dhfr (dihydrofolate reductase), aphIV (hygromycin phosphotransferase), lux (luciferase), uidA (β-glucuronidase). Preferably, the reporter gene is lacZ which codes for β-galactosidase. β-galactosidase can be assayed using the lactose analogue X-gal(5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) which is broken down by β-galactosidase to a product that is blue in color. (See for example Old R.W. & Primrose S.B. Principles of Gene Manipulation. An Introduction to Genetic Engineering, 4th ed. Oxford University Press at pages 63-66 for a discussion of procedures for screening for recombinants).

Additionally, the non-human homologues of genes encoding proteins having Pidd activity can be used to construct a Pidd "knock out" animal which has a defective or altered Pidd gene. For example, a human PIDD cDNA, comprising the nucleotide sequence shown in Figure 10, or a mouse Pidd cDNA appropriate sequence thereof, can be used to clone a murine Pidd gene in accordance with established techniques. A portion of the genomic PIDD DNA (e.g., an exon) can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. The altered *Pidd* DNA can then be transfected into an embryonal stem cell line. The altered *Pidd* DNA will homologously recombine with the endogenous *Pidd* gene in certain cells and clones containing the altered gene can be selected. Cells containing the altered gene are injected into a blastocyst of an animal, such as a mouse, to form aggregation chimeras as described for transgenic animals. Chimeric embryos are implanted as described above. Transmission of the altered gene into the germline of a resultant animal can be confirmed using standard techniques and the animal can be used to breed animals having an altered *Pidd* gene in every cell. Accordingly, a knockout animal can be made which cannot express a functional Pidd protein. Such a knockout animal can be used, for example, to test the effectiveness of an agent in the absence of a Pidd protein.

Although experimental animals used in the preferred embodiment disclosed are mice, the invention should not be limited thereto. It can be desirable to use other species such as rats, hamsters and rabbits.

The transgenic animals of the invention can be used to investigate the effect of PIDD expression and activity or lack thereof and to test other compounds and molecules that can perhaps be used to suppress or restore the p53 apoptosis pathway. The transgenic animals of the invention can also be used to test substances for the ability to prevent, slow or reverse apoptosis. A transgenic animal can be treated with the substance in parallel with an untreated control transgenic animal.

Cells from the transgenic animals of the invention can be cultured using standard tissue culture techniques. In particular, cells carrying the recombinant molecule of the invention can be cultured and used to test substances for the ability to prevent, slow or reverse apoptosis.

EXAMPLES

The following examples describe a novel murine and human p53 regulated gene. The murine cDNA (SEQ ID NO: 13) encodes a protein of 915 amino acids (Pidd, SEQ ID NO:1). The human cDNA (SEQ ID NO: 16) encodes a protein of 910 amino acids (SEQ ID NO: 2). The novel proteins of the invention are referred to as PIDD (for p53 induced protein with a death domain).

Methods

cDNA cloning. RNAimage kits (Genhunter) were used for differential display according to protocols supplied with the kits. Full length cDNA was obtained by 5'-RACE PCR. Sequence analyses and alignments were performed using the NCBI database and SeqWeb on the GCG Wisconsin Package Version 10.

Northern blot analysis. Total RNA was isolated by the single step method using guanidine thiocyanate (18). 10 ug of each RNA sample was run on a denaturing agarose gel and transferred to a positively charged nylon membrane. Hybridization of radioactive probes was done using standard conditions.

Luciferase assay. pGL3-E1bTATA containing a single copy of the p53-binding site from the 5' end of the p21 promoter was obtained from J. Manfredi and is referred to as p21-Luc (14). The p21-derived sequence present in p21-Luc was replaced with the p53 binding consensus sequence from the *Pidd* gene to give rise to Pidd-Luc. Expression vectors carrying either wild type or mutant p53 were co-transfected with the luciferase reporter constructs into p53-negative SAOS2 cells by calcium phosphate Precipitation. 48 hours after transfection, cells were harvested and lysed. Luciferase activity was measured on a LB9507 luminometer using a luciferase assay reagent (Promega) and samples containing equivalent amounts of protein.

Apoptosis assay. K562 cells were co-transfected with expression vectors encoding EGFP and Pidd using the SuPerfect reagent (Qiagen). 48 hours after transfection, cells were harvested and stained with PE-conjugated annexin V and 7-AAD (PharMingen) and analyzed by FACScan flow cytometry. The Percentage of apoptotic cells was calculated based on the proportion of 7-AAD-negative/EGFP-positive cells that were also positive for Annexin V staining.

Growth suppression assay. SAOS2 and K562 cells were transfected with expression vectors encoding Pidd, p53 and the hygromycin-resistance gene as indicated. Hygromycin selection (500 ug/ml for SAOS2 and 100 ug/ml for K562) began 48 hours after transfection. SAOS2 cells were plated on plastic dishes and the K562 cells were selected in medium containing 0.8% methyl cellulose. Hygromycin-resistant colonies were enumerated after 14 days.

Disruption of PIDD expression by antisense oligonucleotides. Four antisense oligonucleotides (ODN) were designed to disrupt mouse and human PIDD expression. ODN-

M76 (5'- AACACTGCAGCCATCACC -3', SEQ ID NO: 25) and ODN-H144 (5'- CTCCACCGTTGCAGCCAT -3', SEQ ID NO: 28) target the translation initiation codons in mouse and human *PIDD* mRNA, respectively. ODN-M603 (5'- GGAAGGTAAGGGCGGGCA -3', SEQ ID NO: 26) and ODN-M837 (5'- AGGTCAGGAGGTTGCTGT -3', SEQ ID NO: 27) target mouse *Pidd* mRNA and were designed using the program OligoWalk in RNAstructure 3.5 (19). All ODNs were synthesized by GIBCO/BRL and purified by HPLC. ODN quality was verified by electrophoresis on denaturing polyacrylamide gels. Antisense ODNs were introduced into cells by reversible streptolysin O (SLO) permeabilization (20) with the exception of ODN-M76, which was introduced by association with lipofectin (GIBCO/BRL) as described by the manufacturer. 5×10^5 cells were mixed with 20 units SLO and 1 μM ODNs on ice. Permeabilization was induced at 37 °C for 5 min and stopped with MEM medium containing 10% fetal bovine serum. Fluorescein isothiocyanate-labelled ODNs were used to show that about 50% of cells took up the ODNs under these conditions. 30 min after ODN uptake, the DP16.1/p53ts cells were transferred to 32 °C to initiate p53-dependent apoptosis, and the OCI/AML-4 cells were exposed to 6 Gy γ radiation to activate endogenous p53 and promote apoptosis. Cells were harvested 16 h later and assayed for apoptosis by Annexin V staining.

Preparation of Polyclonal Antibodies to PIDD

Pidd cDNA was inserted into the bacterial expression vector pET28a and expressed in the BL21 strain *E. coli*. The recombinant His-Pidd fusion protein was induced by IPTG treatment at 37°C for 3 hours and purified by nickel-chelate affinity chromatography. The purified polyhistidine-tagged Pidd fusion protein was dialyzed against PBS and injected into rabbits to generated polyclonal antibodies. The antibodies are effective for immunoprecipitation and for Western blotting. Similar methods are used to generate polyclonal antibodies against other antigenic determinants (epitopes). Peptide-specific antibodies are currently being generated with the following human PIDD derived peptides: (a) human peptide 620-634, ALQRRRDPEQVLLQC (SEQ ID NO: 5) (100% identity with the mouse Pidd 625-639); (b) human peptide 819-833, RIRHEFRDDLDEQIR (SEQ ID NO: 7) (this one has 13 amino acid identical to mouse Pidd 824-838, SEQ ID NO: 6); (c) human peptide 1-14, MAATVEGPELEAAA (SEQ ID NO: 4) (this one has 10 amino acid identical to mouse Pidd 1-15, SEQ ID NO: 3).

Experiments and Results:

Here it is described a novel p53 regulated gene, *PIDD* (p53 induced protein with a death domain), that encodes a protein of 915 amino acids in mice (SEQ ID NO: 1)

and 910 amino acids in humans (SEQ ID NO: 2). The *PIDD* cDNA contains a perfect p53 consensus DNA binding sequence (i.e SEQ ID NO: 18 and 19) upstream of the *PIDD* coding region (SEQ ID NOS: 14 (mouse) and 17 (human)). This sequence element binds to p53 and confers p53-dependent inducibility upon a heterologous reporter gene. Moreover, *PIDD* RNA is induced by ionizing radiation in a p53- dependent manner and the basal level of PIDD RNA is dependent on p53 gene status. Over expression of PIDD inhibits cell growth in a p53-like manner by inducing apoptosis. Antisense inhibition of PIDD expression attenuated p53-mediated apopotosis. These studies define a novel gene whose expression is induced by p53 and that could be an important mediator of p53-dependent growth suppression.

Friend virus-transformed murine erythroleukemia cells that lack endogenous p53 protein expression and express a transfected temperature-sensitive (ts) p53 mutant allele provide a good model to investigate the role of p53 in regulating G1 arrest and apoptosis. The p53ts protein contains valine instead of alanine at amino acid position 135 and behaves as a mutant polypeptide at 37°C and as a wild-type polypeptide at 32°C (5). The inventors have previously shown that the DP 16-1 erythroleukemia cell line expressing p53ts protein (DP 16.1/p53ts) grows well at 37°C. At 32°C, however, these cells arrest in the G1 phase of the cell cycle and undergo apoptosis following expression of the wild-type p53 conformation (6,7). To identify endogenous genes regulated by p53, RNA was prepared from DP16/p53ts cells grown at 37°C and 8 hours after incubation at 32°C, and analyzed by the method of differential display (8). One cDNA fragment, isolated from the differential display method (nucleotides 3559-3938 of Fig. 5, SEQ ID NO: 15) hybridized to a 4.2 kb transcript on Nothern blots that was reproducibly more abundant in DP16/p53ts cells cultured at 32°C compared with parental DP 16 cells grown at 32°C and DP16/p53ts cells grown at 37°C (Fig. 1a). This cDNA fragment was used to obtain full-length cDNA using 5'-RACE PCR. Sequence analysis of multiple cDNAs revealed an open reading frame of 915 amino acids corresponding to a predicted translation product of 101-Kd (Fig. 2a, SEQ ID NO: 1). The initiator methionine (GTGATGG) was situated within a translational initiation consensus sequence (9) and was preceded by an in-frame termination codon 147 bp upstream of the putative initiator methionine. A BLAST search revealed that this was a new molecule with two distinct domains. The N-terminal region encoded 7 tandem leucine-rich repeats (LRRs, SEQ ID NO: 10), a protein interaction motif found in a variety of proteins with diverse functions (10) (Fig. 2b). The C-terminal region (residues 797-877, SEQ ID NO: 8) encoded a death domain with similarity to other death-domain containing molecules (Fig. 2c). This novel protein was named PIDD (for p53 induced protein with a death domain). A homologous and highly conserved human sequence corresponding to the death domain of murine Pidd was identified in the GenBank expressed sequence tag (EST)

data base using the TBLASTN program and is shown in Figure 2c (SEQ ID NO: 9). Related human ESTs were found to be partitioned into a UniGene cluster (Hs.123136) that mapped to the marker SGC30389 on chromosome 11p15.5. EST-derived primers and 5'-RACE PCR were used to obtain full-length human *PIDD* cDNA. The predicted human PIDD protein is shown in Figure 2*a* (SEQ ID NO: 2). The mouse and human PIDD proteins exhibit 81% amino acid sequence identity. Southern blot analysis showed that the *PIDD* gene was probably a single copy gene in the mouse and human genome (data not shown).

Northern blotting analysis indicated that *Pidd* mRNA was expressed predominantly as a single transcript in all adult mouse tissues examined (Fig. 1b). The size of the *Pidd* transcript (4.2 kb) estimated from the gel indicated that the full-length cDNA clone of mouse *Pidd* was isolated. Its expression was relatively higher in spleen, kidney and lung. The low levels of expression in muscle and brain were confirmed by RT-PCR analysis (data not shown). The *Pidd* transcript appeared longer in the testis; a longer transcript was also seen in spleen in addition to the 4.2 kb transcript.

The kinetics of *Pidd* gene induction in DP 16.1/p53ts cells in response to p53 activation at 32°C was studied by Northern blotting (Fig. 1a). *Pidd* mRNA levels increased within 4 hours of p53 activation and remained elevated for 16 hours. This pattern of expression was similar to that of the p53-inducible gene $p21^{WAF1}$ (11). The early induction of *Pidd* expression in p53ts-expressing cells before growth arrest or apoptosis became evident at 6 to 9 hours (7) suggests that *Pidd* induction is not a consequence of p53-mediated growth arrest. The dependency of *Pidd* expression on p53 was investigated further in mouse embryonic fibroblasts (MEFs). Early passage MEFs derived from p53- deficient mice (12) expressed lower levels of *Pidd* mRNA than wild-type MEFs (Fig. 1c). Wild-type MEFs transformed with HPV-16 E7 and H-rasV12 expressed higher levels of *Pidd* mRNA than p53-/- E7/ras MEFs (Fig. 1d). It was also observed that *Pidd* mRNA, like $p21^{WAF1}$ mRNA, was induced in response to γ-irradiation in wild-type MEFs but not in p53-/- MEFs (Fig. 1c).

PIDD mRNA was present in the human wild-type p53-expressing cell line OCI/AML-4 (13) and its level increased after γ-irradiation. Much lower levels were present in the p53-negative cell line K562, and no induction of *PIDD* RNA was observed in these cells after γ-irradiation (Fig. 1*e*). MCF-7 cells were transfected with the same p53ts expression vector used to transfect DP16.1 cells and stable clones were isolated at 37 °C. Endogenous *PIDD* RNA levels rose when MCF-7/p53ts-expressing cells were transferred to 32 °C (Fig. 1*e*). Together these data demonstrate an association between *PIDD* expression and *p53* status in different cell types. Moreover, they demonstrate that the p53-dependent inducibility of *PIDD* mRNA in response to γ-irradiation is conserved in human and mouse cells as well as in different cell lineages.

A putative p53-responsive element was identified in the 5'UTR of mouse and human *PIDD* cDNA (Fig. 3*a*, SEQ ID NO: 18 (murine) SEQ ID NO: 19 (human)). This element matched the published consensus sequence for a p53 DNA binding site RRRCWWGYYYN{0, 13}RRRCWWGYYY (2), in which R is a purine, Y is a pyrimidine and W represents either an A or T residue) in 20 out of 20 positions for mouse *Pidd*; the two half sites in mouse *Pidd* are separated by an insertion of 9 bp. Electrophoretic mobility shift experiments indicated that p53 could bind to the mouse *Pidd* element but not to a scrambled control sequence (Fig. 3*b*). To determine whether p53 could activate transcription of a minimal promoter containing the mouse *Pidd* p53-binding sequence, a double stranded synthetic oligonucleotide containing one copy of this element was inserted into the luciferase reporter vector pGL3-E1bTATA (designated Pidd-Luc). Two additional reporter plasmids served as controls: one in which the *Pidd* derived sequence was scrambled (C-Luc) and another containing the p53-binding site from the $p21^{WAF1}$ promoter (14) (p21-Luc). SAOS2 cells, which harbour a homozygous deletion of the *p53* locus and do not produce p53 protein (15), were transfected with these reporter plasmids along with plasmids expressing either wild-type p53 or mutant p53 (p53 V143A) (16). Transfection of p53 enhanced luciferase expression from Pidd-Luc and p21-Luc but not from C-Luc. p53 V143A had no activity in these assays (Fig. 3*c*). Hence, the p53-binding site present in *Pidd* conferred p53-dependent transcriptional activation on a minimal promoter. Together, these experiments indicate that *Pidd* expression is associated with wild-type but not mutant p53 expression and that *Pidd* represents a novel p53 target gene whose expression is likely directly induced by p53.

Reintroduction of wild-type p53 suppresses the growth of p53-null cells. If Pidd plays a role in mediating the tumour growth inhibition of p53, one might expect it to have a growth suppressive role of its own. To address this possibility, the ability of mPidd to inhibit the growth of p53-deficient SAOS2 and K562 cells in a colony assay after co-transfection with a plasmid encoding resistance to hygromycin was tested. Cells were placed under hygromycin selection two days after transfection and the drug-resistant colonies were enumerated approximately two weeks later. In multiple, independent experiments, mPidd expression resulted in substantial growth suppression similar to that observed with p53 (Fig. 4*a*).

To determine whether the growth suppression might be due to apoptosis, K562 cells were transiently transfected with a plasmid encoding enhanced green fluorescent protein (EGFP) together with plasmids encoding either p53 or mPidd. After 48 hours, apoptosis was assessed by Annexin V staining of the EGFP-positive population by fluorescence-activated cell sorting. As expected, p53 gave a significant increase in the

number of cells undergoing apoptosis compared with cells transfected with empty vector (Fig. 4*b*). Pidd induced apoptosis in K562 cells to a similar extent as p53 (Fig. 4*b*).

The inventors have also identified and characterized the human PIDD cDNA in a human leukemia cell line and in human fibroblasts, although any human cells could be used. The following primers (ESTs) were used to identify and characterize the human nucleic acid sequence encoding PIDD:

1. Genebank accession No.: AA326188 (matches the PIDD sequence at nucleotides 568-741 of Figure 6, 1332-1502 of Figure 10 and SEQ ID NO: 29);
2. Genebank accession No.: R13288 (matches nucleotides 812-1210 of Figure 6, 1576-1974 of Figure 10, and SEQ ID NO: 30); and
3. Genebank accession No.: AA631067 (matches nucleotides 1591-1944 of Figure 6, 2525-2878 of Figure 10 and SEQ ID NO: 31).

Much the same techniques as described for the murine Pidd were used. The partial nucleic acid sequence encoding human PIDD is shown in Figure 6. The full nucleic acid sequence is shown in Figure 10. Figure 8 shows the alignment of the human *PIDD* nucleic acid sequence with the murine *Pidd* nucleic acid sequence. Figure 7, illustrates the percent homology between the cDNA sequences of the death domains from mouse *Pidd*, human PIDD, Human RIP, Human FADD, Human RAIDD, and human DAPK.

The bar graph of Figure 9 illustrates that PIDD is involved in p53 mediated apoptosis. As described above, endogenous PIDD expression was inhibited using antisense oligonucleotides and the investigators determined whether PIDD downregulation could affect p53-dependent apoptosis in DP16.1/p53ts cells. As described above, cells were exposed to three different 18-base antisense oligonucleotides directed to different regions of the m*Pidd* mRNA sequence and incubated at 32 °C for 16 hours prior to assessment of apoptosis. Appropriate controls (no oligonucleotide, addition of sense or mismatched oligonucleotides, which had the same base composition as the antisense oligonucleotides but a scrambled nucleotide sequence) were used in parallel. Oligonucleotides were delivered to cells and their uptake monitored, as previously described. The three antisense oligonucleotides significantly attenuated p53-dependent apoptosis although the control oligonucleotides gave no significant effects (Fig. 9).

Figure 9 also illustrates the affect of PIDD on γ-radiation-induced apoptosis in human cells. OCI/AML-4 cells were exposed to 6 Gy γ–radiation in the presence or absence of an antisense (H144, SEQ ID NO: 28), sense or mismatched oligonucleotide. Apoptosis was measured 16 hours after irradiation. A significant attenuation of apoptosis was seen with the antisense oligonucleotide although the control

oligonucleotides gave no effect (Fig. 9). Taken together, these results indicate that PIDD is involved in p53-mediated apoptosis and that PIDD is required for apoptosis in more than one cell type.

Figure 11, illustrates that polyclonal antibodies can be used to determine PIDD expression. Polyclonal antibodies to PIDD were made as described above and made in accordance with the method described above. The antibody to the mouse PIDD was used to isolate PIDD protein in a sample by immunoprecipitation. The sample was then run on a gel and a Western blot was probed with the antibody to determine PIDD expression. Further antibodies can be made to different epitopes of PIDD. Antibodies can be made to peptide fragments which are specific thereto. The peptide fragments can be conserved or not conserved among individuals or species. Example, of suitable peptide sequences which can be used to make antibodies include peptides from the LRR, or Death Domain regions, or if desired to sequences which are conserved among species. Examples of suitable peptides include: SEQ ID NO: 4, 5, or 7

In conclusion, the present inventors have identified a novel gene that is regulated by p53. The ability of PIDD, like p53, to suppress the growth of p53-deficient cells and to promote apoptosis indicates that PIDD likely functions downstream of p53. Antisense inhibition of PIDD expression attenuated apoptosis in response to p53 activation and DNA damage. It is suggested that PIDD is an essential component of the DNA damage/stress response pathway that connects p53 to apoptosis. Although a number of candidate p53-effector molecules have been reported, the mechanism through which activated p53 promotes apoptosis remains unclear (17). Identification of molecules that interact with PIDD through its LRR domain or its death domain will provide additional components of the p53 apoptosis pathway.

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated to those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. We claim all modifications coming within the scope of the following claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Below full citations are set out for the references referred to in the specification and detailed legends for the figures are provided.

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WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule having a sequence encoding a p53 induced protein with a death domain (PIDD) or fragment thereof.

2. An isolated nucleic acid molecule of claim 1 having a sequence shown in SEQ ID NO: 13 or 16.

3. An isolated nucleic acid molecule having a sequence shown in SEQ I NO: 20, 21, 22, 23 or 24.

4. An isolated nucleic acid molecule having a p53 binding sequence shown in SEQ ID NO: 18 or 19.

5. An isolated nucleic acid molecule having a sequence shown in SEQ ID NO: 14, 15 or 17.

6. An isolated nucleic acid molecule having a sequence shown in SEQ ID NO: 29, 30 or 31.

7. An isolated nucleic acid molecule as claimed in claim 1, which comprises: (a) a nucleic acid sequence as shown in SEQ ID NO: 13 or 16, wherein T can also be U; (b) a nucleic acid sequence complementary to (a); (c) a nucleic acid sequence which is at least 85% homologous to (a) or (b); (d) a nucleic acid sequence which differs from any of the nucleic acid molecules of (a) to (c) in codon due to the degeneracy of the genetic code; or, (e) a fragment of the nucleic acid sequence of (a), (b), (c) or (d) that is at least 15 bases and which will hybridize to (a), (b), (c) or (d) under stringent hybridization conditions.

8. A recombinant molecule adapted for transformation of a host cell comprising a nucleic acid molecule as claimed in claim 7 and an expression control sequence operatively linked to the DNA segment.

9. A transformed host cell including a recombinant molecule as claimed in claim 8.

10. A method for preparing a PIDD or a fragment thereof utilizing an isolated nucleic acid molecule as claimed in claim 1.

11. A method for preparing a PIDD utilizing a isolated nucleic acid molecule as claimed in claim 2.

12. A PIDD polypeptide.

13. The PIDD as claimed in claim 12 having one of the amino acid sequences shown in SEQ ID NO: 1 or 2 or a peptide encoded by the nucleic acid sequence of SEQ ID NO: 13 or 16, or a fragment thereof.

14. An antigenic isolated PIDD as claimed in claim 13 or a fragment comprising at a least 14 amino acid sequence thereof.

15. An antigenic isolated fragment of PIDD as claimed in claim 14 having a amino acid sequence of SEQ ID NO: 3, 4, 5, 6, or 7.

16. An isolated peptide having a PIDD leucine rich repeat sequence of SEQ ID NO: 10 or 11.

17. An isolated peptide having PIDD or death domain sequence of SEQ ID NO: 8 or 9.

18. An isolated peptide having a sequence of SEQ ID NO: 12.

19. An antisense nucleic acid molecule of the nucleic acid molecules of any one of claims 1 to 7.

20. An antisense nucleic acid molecule of claim 19 which is directed to the region preceding the initiation codon, a region including the initiation codon, all or part of the PIDD amino acid coding region of the nucleic acid molecule or an unconserved region thereof.

21. An antisense nucleic acid molecule selected from the group consisting of SEQ ID NOS: 25, 26, 27 and 28.

22. A monoclonal or polyclonal antibody specific for an epitope of the polypeptide as claimed in claim 12.

23. A monoclonal or polyclonal antibody specific for an epitope of the polypeptide as claimed in claim 13.

24. A monoclonal or polyclonal antibody specific for an epitope of the polypeptide having an amino acid sequence of any one SEQ ID NOS: 3 to 7.

25. A method for detecting PIDD in a sample comprising contacting the sample with an antibody specific for an epitope of the polypeptide as claimed in claim 13 which is capable of being detected after it becomes bound to PIDD in the sample.

26. A method for detecting PIDD in a sample comprising contacting the sample with a monoclonal or polyclonal antibody as claimed in claim 23 which is capable of being detected after it becomes bound to the PIDD protein in the sample.

27. A method for detecting the presence of a nucleic acid molecule as claimed in claim 1 in a sample, comprising contacting the sample with a nucleotide probe capable of hybridizing with the nucleic molecule, to form a hybridization product, under conditions which permit the formation of the hybridization product, and assaying for the hybridization product.

28. A method for detecting the presence of a nucleic acid molecule as claimed in claim 1, or a predetermined oligonucleotide fragment thereof in a sample, comprising treating the sample with primers which are capable of amplifying the nucleic acid molecule or the predetermined oligonucleotide fragment thereof in a polymerase chain reaction to form amplified sequences under conditions which permit the formation of amplified sequences, and assaying for amplified sequences.

29. A kit for detecting PIDD by assaying for PIDD in a sample comprising an antibody specific for an epitope of the purified and isolated polypeptide as claimed in claim 12, reagents required for binding of the antibody to PIDD in the sample, and directions for its use.

30. A kit for detecting PIDD by assaying for a PIDD in a sample comprising a monoclonal or polyclonal antibody as claimed in claim 22, reagents required for binding of the antibody to PIDD in the sample, and directions for its use.

31. A kit for detecting the presence of a nucleic acid molecule as claimed in claim 1 in a sample comprising a nucleotide probe capable of hybridizing with the nucleic acid molecule, reagents required for hybridization of the nucleotide probe with the nucleic acid molecule, and directions for its use.

32. A method for treating or preventing a disease in animal associated with the expression of PIDD comprising administering an effective amount of an agent that inhibits PIDD expression or function to an animal in need thereof.

33. A method according to claim 32 wherein the agent is an antibody according to claim 22.

34. A method according to claim 32, wherein the agent is an antisense nucleic acid molecule according to any one of claims 19-21.

35. A method according to claim 22, wherein the disease is an autoimmune disease, neurodegenerative disease, or stroke.

36. A method for treating or preventing a disease in animal associated with the expression of PIDD comprising administering an effective amount of an agent that activates or simulates PIDD activity to an animal in need thereof.

37. A method of claim 36, wherein the disease is cancer, dysplasia, or Li-Fraumeni Syndrome.

38. A method for treating or preventing a disease in an animal associated with deficient or insufficient apoptosis comprising administering effective amount of an agent that activates or simulates PIDD activity to an animal in need thereof.

39. A method of claim 38, wherein the disease is cancer or dysplasia, or Li-Fraumeni Syndrome.

40. A PIDD knockout transgenic non-human animal.

41 The transgenic animal of claim 30 , wherein the animal is a mouse.

42. A method of identifying a modulator of PIDD expression or activity comprising: incubating a potential modulator with a cell comprising an expressable *PIDD* gene and then monitoring the effect on PIDD expression or activity.

43. A method of claim 42 where PIDD expression or activity is monitored by an apoptosis assay.

44. The method for of claim 42 wherein the potential modulator suppresses PIDD induced apoptosis.

45. The method of claim 44 where the potential modulator is an antisense nucleic acid molecule of all or part of SEQ ID NO: 13 or 16.

46. The method of cliam 44 wherein the potential modulator is an antibody.

47. The method of claim 42 where the modulator stimulates or stimulates PIDD related activity.

48. The method of claim 47 wherein the PIDD related activity is apoptosis.

49. A use of anyone of the peptides of claim 12 -18 for preventing or treating a condition associated with *PIDD* expression or PIDD activity.

50. A use of anyone of the peptides of claim 12-18 for the manufacture of a medicament for preventing or treating a condition associated with *PIDD* expression or PIDD activity.

51. A use of a nucleic acid molecule of any of claims 1-6 for preventing or treating a condition associated with *PIDD* expression or PIDD activity.

52. A use of an antisense nucleic acid molecule of any of claims 19-21 for preventing or treating a condition associated with *PIDD* expression or PIDD activity.

53. A use of an anitbody of anyone of claims 22-24 for preventing or treating a condition associated with *PIDD* expression or PIDD activity.

FIGURE 1a

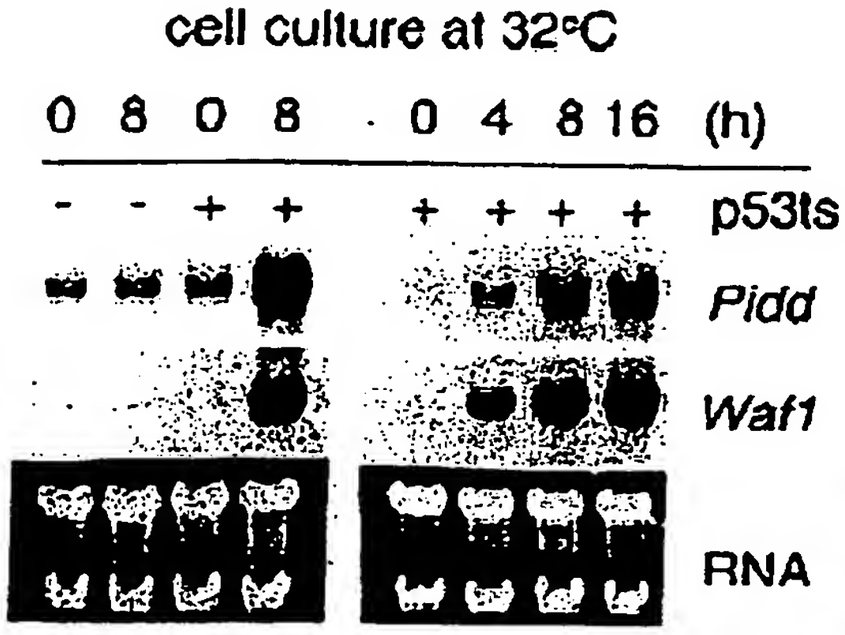

FIGURE 1b

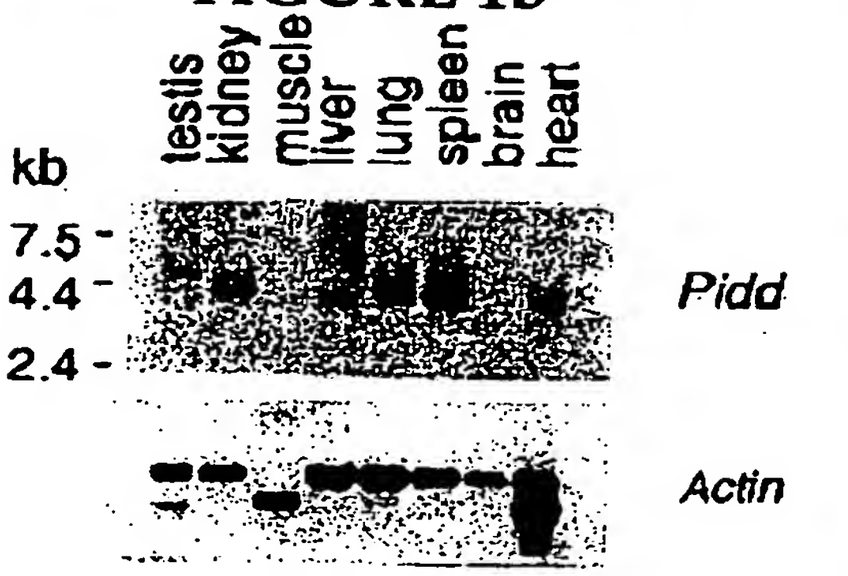

FIGURE 1c

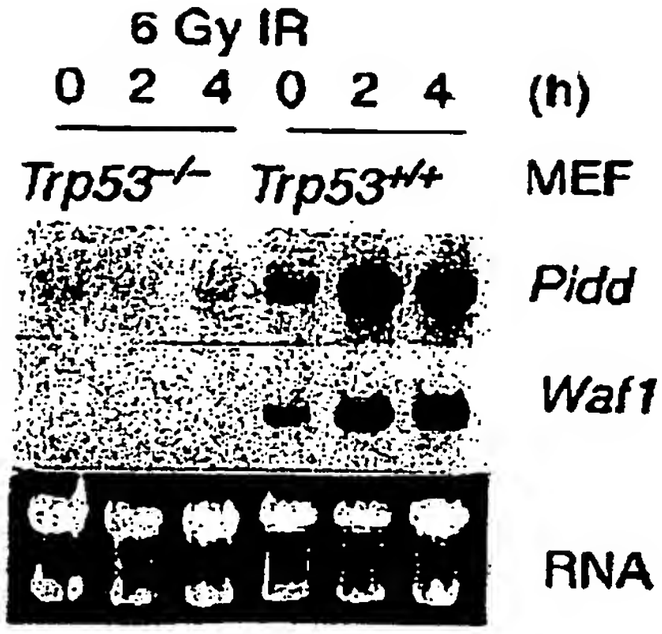

FIGURE 1d

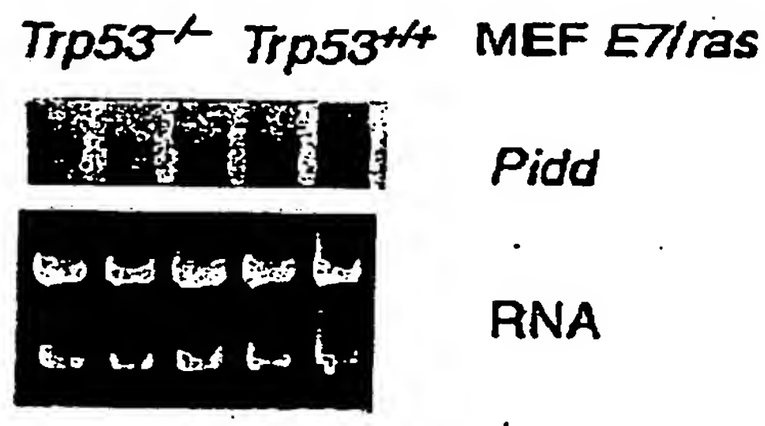

FIGURE 1e

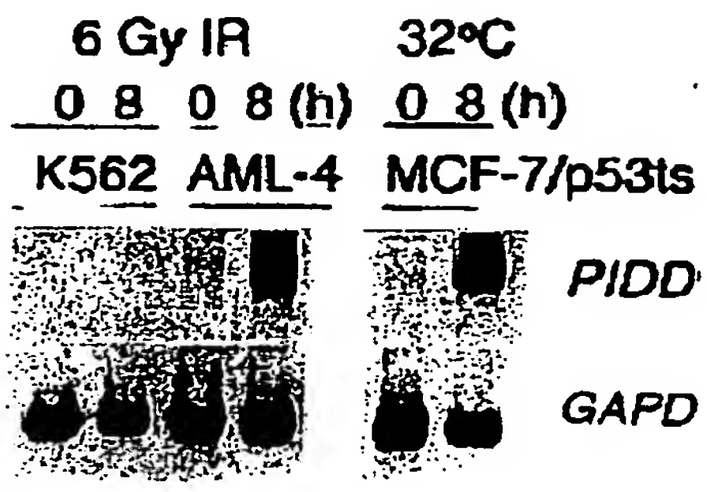

FIGURE 2a

```
mouse   MAAVLEGQEPEETAAAAEDAATSTLEAVDAGPGA-PFLPAGNQLNLDLRPGGCHRLQYLC  59
human   ...TV..P.-L.A....G..S----.DS...SR.L...-G..R.S...Y....QQ.LH..  54

mouse   SQQPPQLLQVEFLRLSTHEDPQLLDDTLAKVPWSLLRLRSLVLKGGQSRGALGACLHGTL  119
human   V...L.....................EA...QL.Q..SC..........R.DT.....R.A.  114

mouse   TTLPAGLSDLACLAHLDLSFNRLETLPTCVPELHGLDALLLSHNHLSELPHALGALPALT  179
human   .N......G..H.........S.....A..LQMR..G.......C...............  174

mouse   FLTVTHNRLERLPLTLGSLSTLQRLDLSENLLDTIPSEIGNLRSLSELNLASNRLQSLPA  239
human   .........QT..PA..A..........Q.....L.P...G.G..L...............  234

mouse   SLAGLRSLRLLVLHSNLLTSVPTGLVHLPLITRLDLRDNRLRDLPAELLDAPFVRLQGNP  299
human   ...................A...AD.AR...L........Q.....P..............  294

mouse   LGEASPAPPSPPDISQVPEMPRLLLTSDLDSFLVTPHGCSVTLACGVRLQFPAGATTTPV  359
human   ......DA..S.VAALI......F........P...R...................A..I  354

mouse   TIHYRLWLPEPGLVSLGPHDFLLSSVLELQPHGVAFQQDVSLWLLFVPPRVRRCREVVVR  419
human   ..R...L.......P.....A...H..............G.....T..QA.........  414

mouse   TRSNNTWNDLETQLEEEAPKRLWARCQVPHFSWFLVVLRPVSNTCLLPPEGALLCSSGHP  479
human   ..ND.S.G....Y......Q....H............S.....A..V....T........  474

mouse   GVRVTFPPGVTEEPRQVSMQVVHMAGLELRTLLEESEASVSPLLCLSQSGPPSFLQPVTV  539
human   ..K.I....A.....R......R...R..QA..G.P..A.....................  534

mouse   QLPLPPGVTGFSLDRSHLHLLYRTPLTTTWDDITTQVALEFTHLYARFQVTHFSWYWLWY  599
human   .....S.I..L.....R.....WA.PAA......A..V..L...................  594

mouse   TTKTCVGGLARKAWERLRLHRVNLIALQRRRDPEQVLLQCLPRNKVDATLSRLLVRYRGP  659
human   ...N.....................................................R...E.....  654

mouse   EPSETVEMFEGEKFFAAFERGIDVDADRPDCVDGRICFVFYSHLKNVKEVYITTALDREA  719
human   ...D........E.....................E...................V..T.....  714

mouse   QDVRGQVSFYRGSLPVEVPAEAEAARQRKGTDALWMATLPIKLPRLRGAQGSGQGTDFSL  779
human   .A..........AV..R..E..........A.................SE.PRR.AGL..  774

mouse   MPLNLGDAETGFLTQSNLLSVASRLGPDWPAVALHLGMPYHKLQRIRHEFRDDLDGQVRH  839
human   A.....................G...L..........VS.REV............E.I..  834

mouse   MLFSWAERQTGQPGAVGHLVQALEQSDRRDVAEEVRALELGRHKYQDSIRRTGLAPEDS  899
human   .........A.......L..........Q........V.....R........M....K.P  894

mouse   TLPGTSASQTPESAQA  915
human   A...S..P.P..P...  910
```

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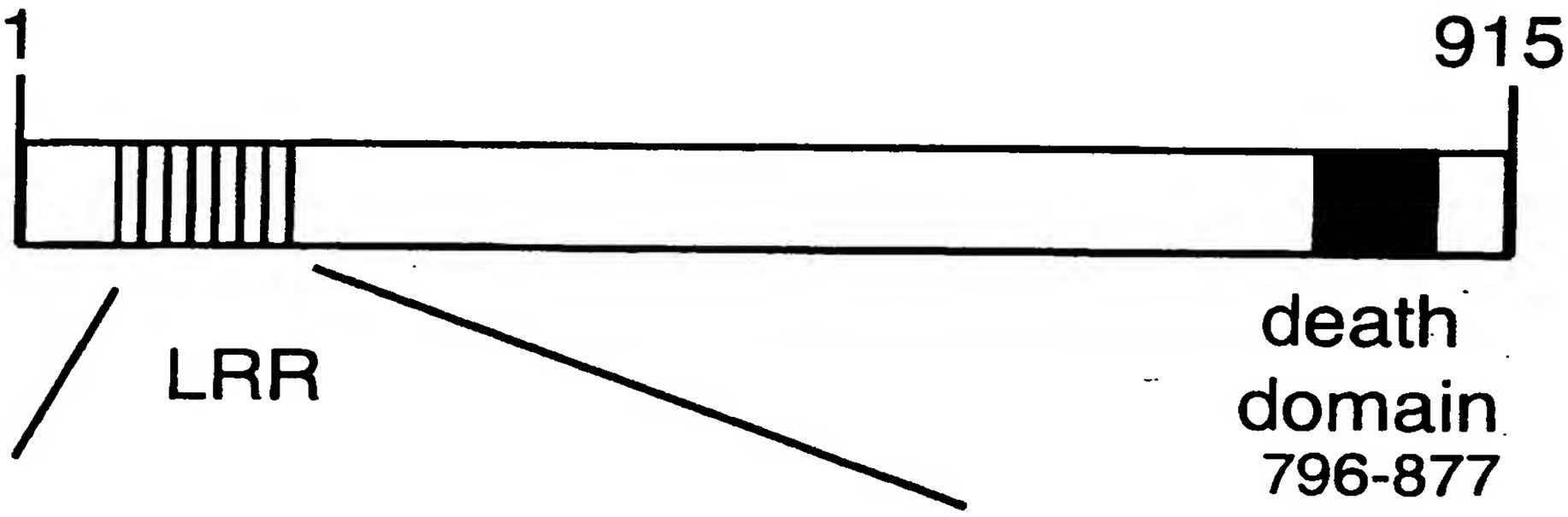
FIGURE 2b




```
.l..L.l..N.L..lP..l..l.
CLAHLDLSFNRLETLPTCVPELH 131-153
GLDALLLSHNHLSELPEALGALP 154-176
ALTFLTVTHNRLERLPLTLGSLS 177-199
TLQRLDLSENLLDTIPSEIGNLR 200-222
SLSELNLASNRLQSLPASLAGLR 223-245
SLRLLVLHSNLLTSVPTGLVHLP 246-268
LITRLDLRDNRLRDLPAELLDAP 269-291
```

FIGURE 2c

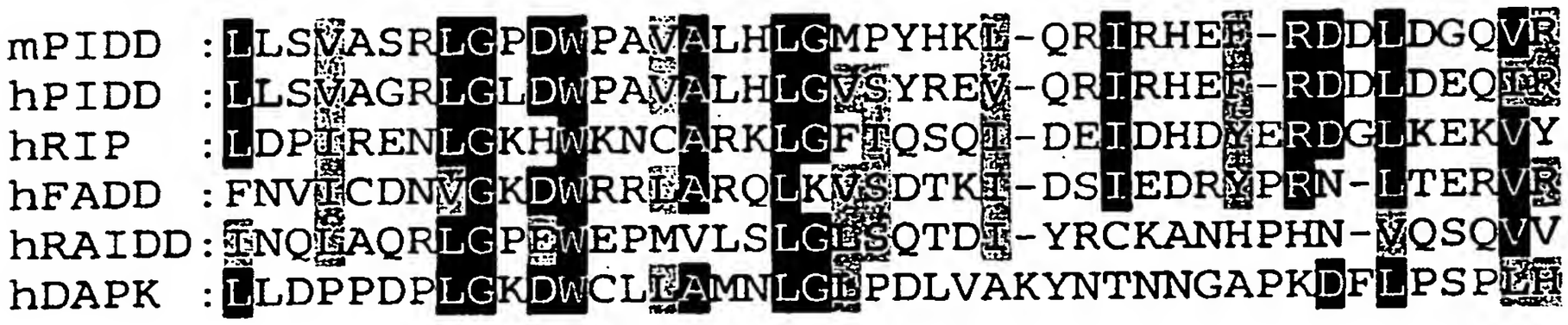

mPIDD :LLSVASRLGPDWPAVALHLGMPYHKL-QRIRHEE-RDDLDGQVR
hPIDD :LLSVAGRLGLDWPAVALHLGVSYREV-QRIRHEE-RDDLDEQIR
hRIP :LDPTRENLGKHWKNCARKLGFTQSQT-DEIDHDYERDGLKEKVY
hFADD :FNVLCDNVGKDWRRLARQLKVSDTKI-DSIEDRYPRN-LTERVR
hRAIDD:INQLAQRLGPEWEPMVLSLGESQTDI-YRCKANHPHN-VQSQVV
hDAPK :LLDPPDPLGKDWCLLAMNLGLPDLVAKYNTNNGAPKDFLPSPLH
mPIDD :HMLFSWAERQTGQPGAVGHLVQALEQSDRRDVAEEVRAL
hPIDD :HMLFSWAERQAGQPGAVGLLVQALEQSDRQDVAEEVRAV
hRIP :QMLQKWVMREGIKGATVGKLAQALHQCSRIDLLSSLIYV
hFADD :ESLRIWKNTEK-ENATVAHLVGALRSCQMNLVADLVQEV
hRAIDD:EAFIRWRQR-FGKQATFQSLHNGLR-AVEVDPSLLEHMI
hDAPK :ALLREWT---TYPESTVGTLMSKLRELGRRDAADLELKA

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FIGURE 3a

-54 -26

(M) **AGGCTTGCTT**GGCAAGCTG**GGACATGTCT**

(H) **AGGCCTGCCT**·GCGTGCTG**GGACATGTCT**

FIGURE 3b

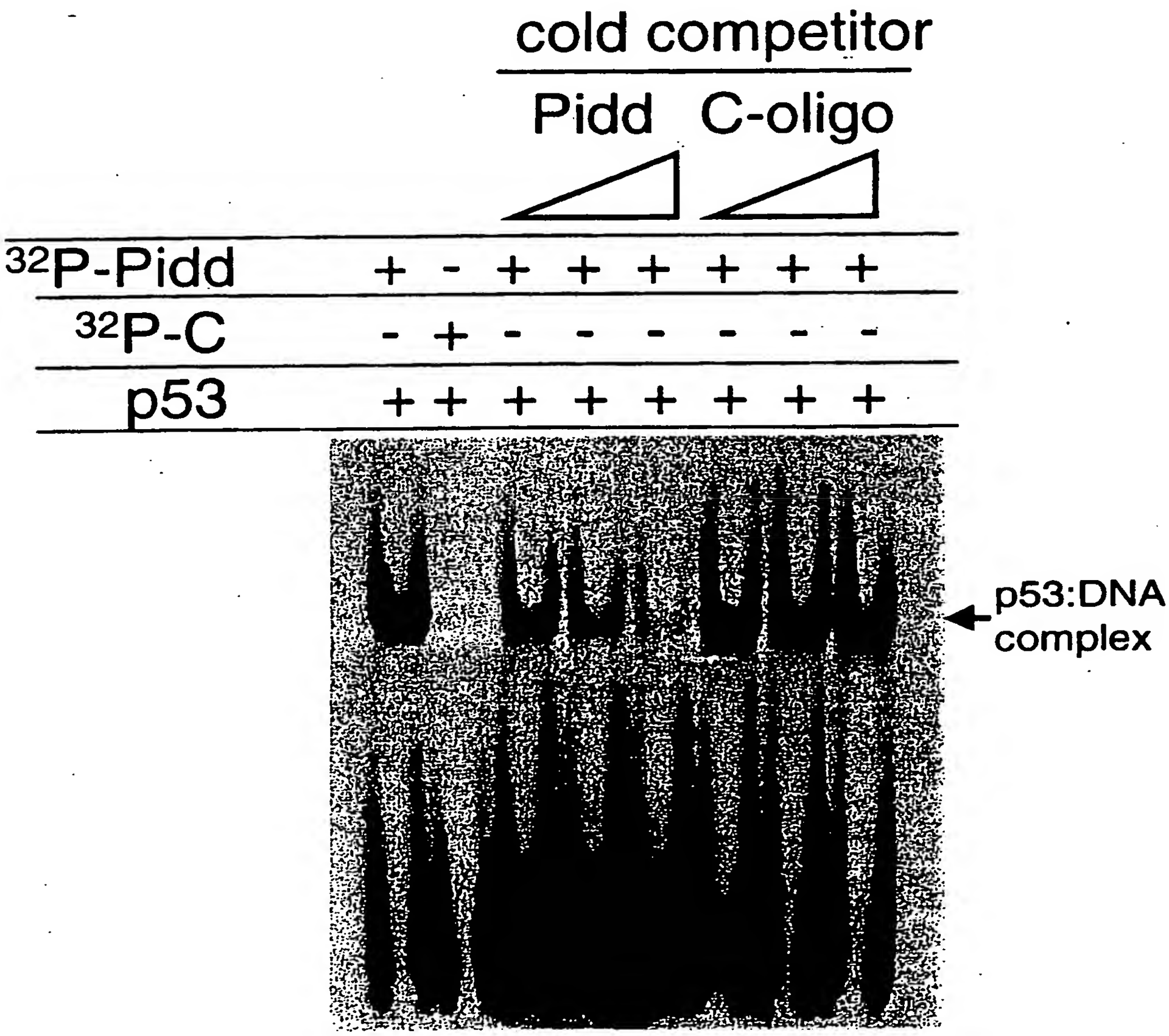

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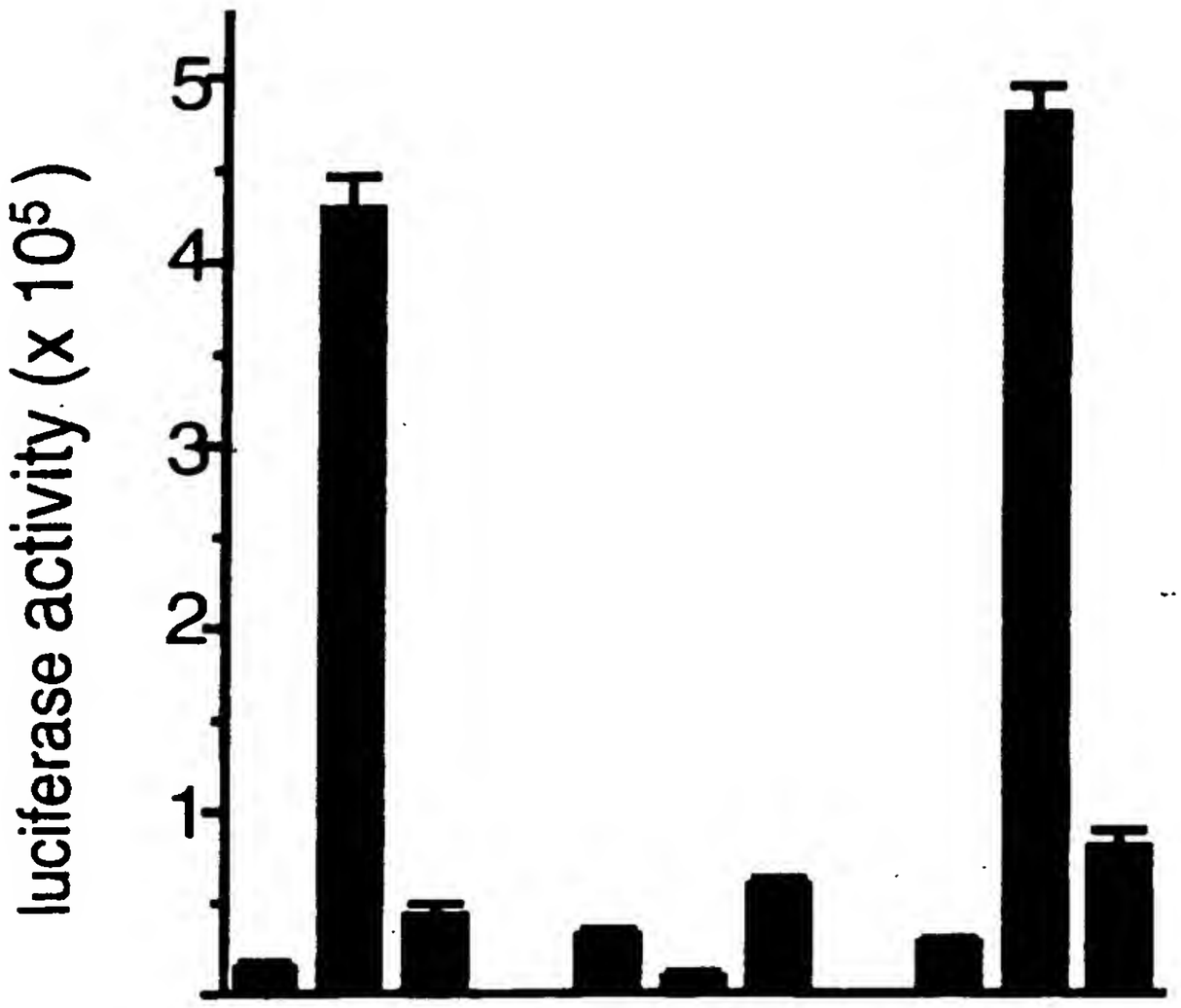
FIGURE 3c



p21-Luc	+	+	+								
C-Luc					+	+	+				
Pidd-Luc									+	+	+
pCDNA3	+				+				+		
p53wt		+				+				+	
p53V143A			+				+				+

FIGURE 4a

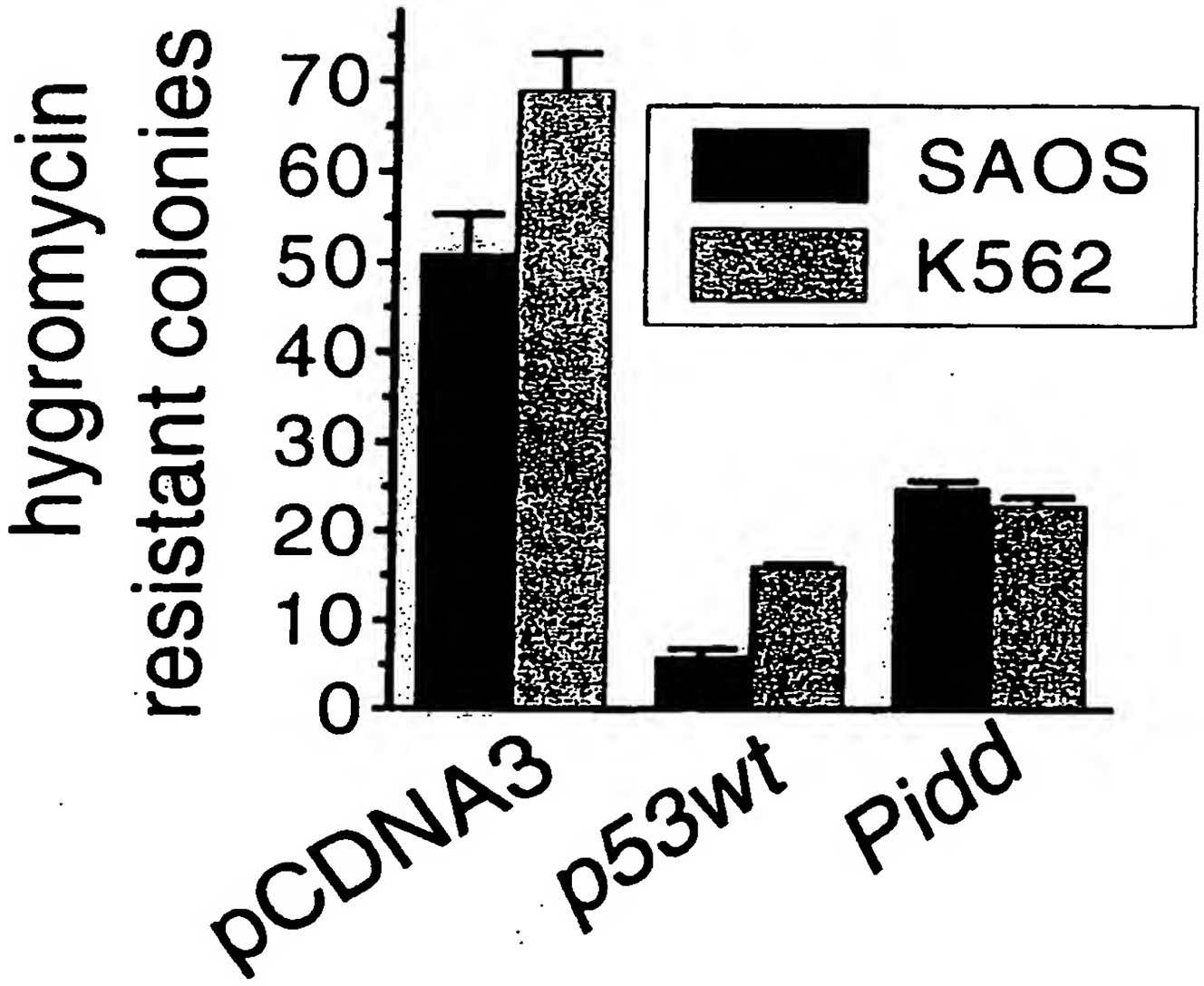


FIGURE 4b

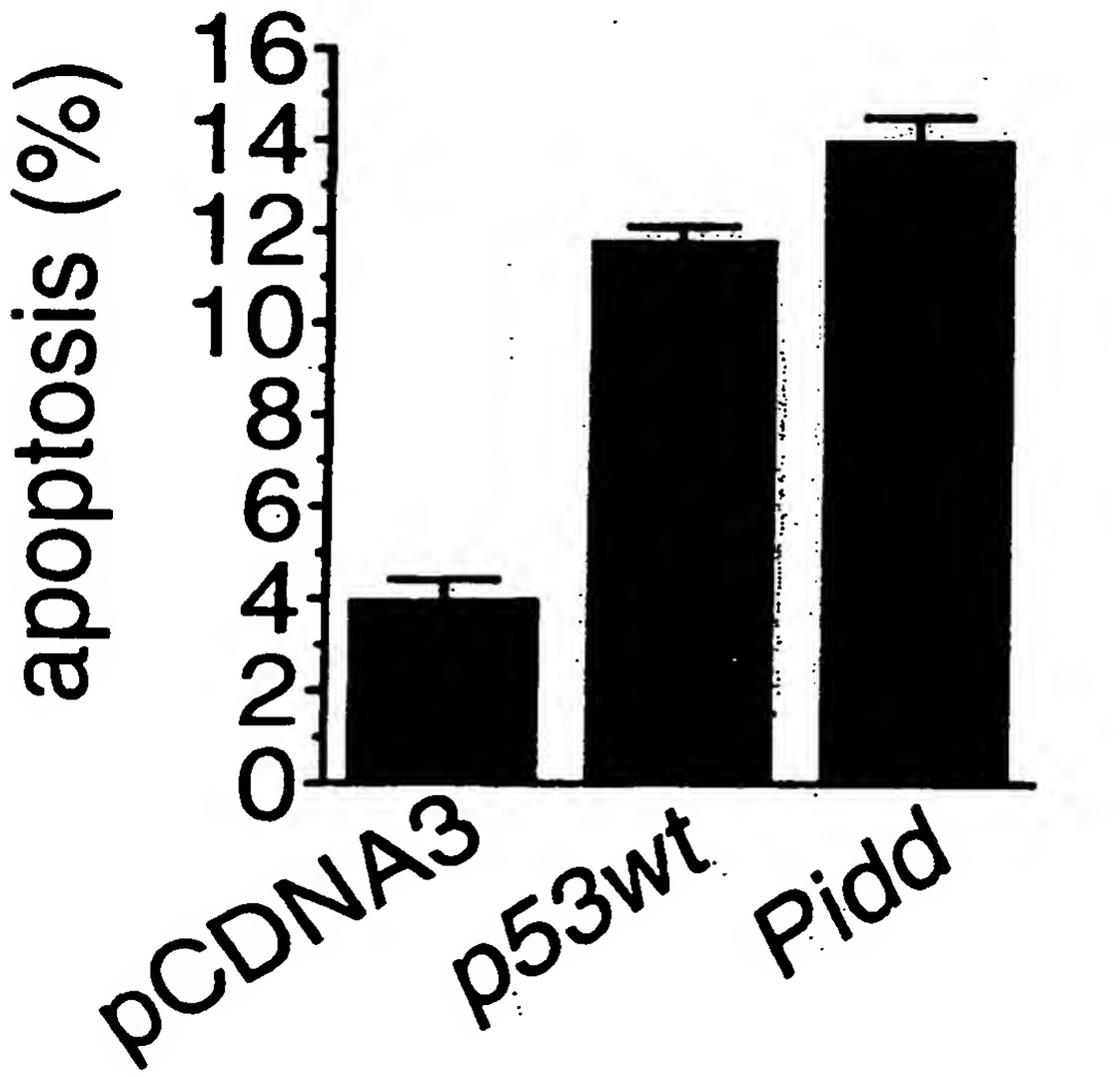


FIGURE 5

Mouse Pidd cDNA sequence

```
   1  GAGCGCGCCT AGACTGGCGG GGAGATCGGC GTGGCAGGTA CTCAATGGGA
  51  GGCGGGAAGT TTGCTCCAGA GGCGGGACCA AAGCGATTCC TCACCTACCG
 101  AGACCCCCTC TGCTTTCCCC ATTTGACGGA GGTGTGAATT GGGAATTGTG
 151  GCTCAAGAGT GTCTACTAGG GACCGCGATA TGGGACCCTA GCACAAGAAC
 201  CCCAAAGTCC TTTTGGCTCC CCTTCGCACT GTTTACTGAC CTTCTCGGGC
 251  ACAGTGAACG TCTGCAGTGG AAGAAGGTTC TGGGTTCTCC CCATGCATTT
 301  GGGTGTGAAG ATAGAAAGTG TGTCCTTATT TCCAGAGCCC TGTAGGGATC
 351  AGGGATCACT TGGGCAGATT CCAGAATTGA GGTGGAAACC CTCTCCTTGT
 401  CCTTGATCTA AGCAGAGTTC TCCAAGCTAT GCTGTTCAGA GTCGCCCTGC
 451  CTGGTGCCCC TCCCAGGCAG AAGGATCCAG CACAGATGCA AGCCGTAGGG
 501  CTGCAGTGAC CACTCCACTC ACGACCCCTT GTGCCCAGGG TTCTTGGGCT
 551  GGTGGTCTCA AGAGCTCCCG GGTTTCCAAG CTGAGTTATT GTTGCTCTGA
 601  CCTGGAGGGG GAGCTGGTCC CCTACAGCCT ACTCCTCCCC CTGCTTCTCC
 651  AGCTTCTATC AGCCTTGCCT AAGCAGGCTT GCTTGGCAAG CTGGGACATG
 701  TCTGGTCCCC GAGGACCTTC CATGGGTGAT GGCTGCAGTG TTGGAGGGGC  Met1
 751  AGGAGCCGGA GGAAACTGCA GCTGCTGCAG AGGATGCTGC AACATCCACC
 801  TTGGAGGCTG TGGATGCCGG GCCTGGAGCA CCTTTTCTCC CGGCTGGGAA
 851  CCAGTTGAAC TTGGACCTAC GCCCAGGGGG CTGCCATCGG CTACAGTACC
 901  TGTGTTCCCA GCAGCCCCCA CAGCTGCTGC AAGTGGAGTT TTTGCGGCTC
 951  AGCACCCATG AGGACCCTCA GCTGCTGGAT GACACCCTAG CCAAGGTTCC
1001  GTGGAGTCTG CTGCGCCTTC GCTCCCTGGT CCTCAAAGGT GGCCAGAGCC
1051  GGGGTGCCCT GGGTGCCTGC CTCCATGGAA CCCTGACCAC TCTGCCTGCT
1101  GGCCTGAGTG ATTTGGCCTG CCTGGCTCAC CTAGACCTGA GCTTCAACAG
1151  GCTGGAGACG CTGCCTACCT GTGTCCCGGA ACTGCACGGC CTGGATGCGT
1201  TGCTGCTTTC TCACAACCAT CTCTCAGAGC TGCCCGAGGC CCTGGGGGCA
1251  CTGCCCGCCC TTACCTTCCT CACCGTGACA CACAACCGCC TAGAAAGACT
```

FIGURE 5 (CONT'D)

1301 ACCCCTAACA CTCGGGTCCC TGTCCACCCT TCAGCGTCTC GATCTCTCTG

1351 AGAACCTTCT AGACACCATA CCCTCTGAGA TTGGAAACCT GCGCAGCCTC

1401 AGTGAGCTCA ATCTGGCCTC CAACCGGCTG CAGAGTCTCC CAGCTTCCCT

1451 CGCGGGGCTG CGGTCCCTGC GGCTCCTTGT TCTGCACAGC AACCTCCTGA

1501 CCTCAGTGCC CACTGGCCTG GTCCACCTGC CACTGATCAC TCGGCTTGAC

1551 CTGAGGGACA ACCGGCTCCG AGACCTGCCT GCTGAGTTAC TAGACGCTCC

1601 CTTTGTGCGC CTGCAGGGGA ACCCTCTGGG CGAGGCCTCT CCAGCACCCC

1651 CAAGTCCCCC AGACATATCC CAGGTTCCAG AAATGCCCAG GCTATTGCTG

1701 ACCTCAGATT TGGACAGCTT CCTTGTGACT CCCCATGGCT GTTCTGTGAC

1751 CCTGGCCTGT GGTGTCCGCC TACAGTTTCC AGCAGGAGCC ACCACCACGC

1801 CTGTCACCAT CCACTATCGA CTCTGGTTGC CCGAGCCAGG CCTGGTCTCT

1851 CTGGGACCTC ATGACTTCCT GCTTAGTAGT GTCCTGGAGC TGCAGCCCCA

1901 TGGGGTGGCT TTCCAGCAAG ATGTGAGCTT ATGGCTGCTC TTTGTCCCTC

1951 CACGAGTCCG TCGCTGTCGT GAGGTAGTTG TGAGGACACG GAGTAACAAC

2001 ACCTGGAATG ACCTAGAGAC CCAACTGGAG GAAGAGGCAC CCAAGAGGCT

2051 CTGGGCTCGC TGCCAGGTGC CCCACTTCTC CTGGTTCCTG GTCGTTTTAC

2101 GCCCAGTATC CAACACTTGC CTTTTGCCAC CAGAGGGAGC ACTTCTCTGC

2151 TCCTCAGGTC ATCCTGGGGT CAGAGTCACT TTTCCCCCTG GGGTTACAGA

2201 AGAGCCTCGG CAAGTCTCCA TGCAGGTAGT GCATATGGCT GGACTAGAGC

2251 TGAGAACCCT TCTGGAAGAA TCAGAGGCAT CAGTAAGCCC TCTGCTGTGC

2301 CTTTCACAGA GCGGCCCCCC CAGCTTCCTA CAACCTGTCA CTGTGCAGTT

2351 GCCCCTGCCT CCTGGCGTTA CAGGCTTCAG TCTAGACCGC TCCCACCTGC

2401 ATCTGCTCTA CCGAACACCC CTGACAACCA CCTGGGATGA TATCACCACT

2451 CAGGTGGCAC TGGAATTCAC CCACCTGTAT GCACGCTTCC AGGTCACGCA

2501 CTTCTCCTGG TACTGGCTCT GGTATACCAC CAAAACCTGT GTGGGGGGCC

2551 TGGCCCGGAA GGCCTGGGAA CGGCTGCGAC TGCACCGTGT GAATCTCATT

2601 GCACTGCAGA GGCGCCGAGA CCCTGAGCAG GTTCTGCTGC AGTGCCTGCC

FIGURE 5 (CONT'D)

```
2651  ACGGAACAAG GTAGATGCCA CCCTGAGTCG GCTGCTGGTT CGCTACCGTG
2701  GCCCTGAACC CTCTGAGACT GTGGAGATGT TTGAGGGTGA GAAGTTCTTT
2751  GCAGCCTTTG AGCGGGGCAT TGATGTAGAT GCAGACCGTC CAGACTGTGT
2801  GGATGGCAGA ATCTGCTTTG TCTTCTATTC ACACCTGAAG AACGTAAAGG
2851  AGGTATACAT CACCACAGCC TTGGACCGGG AAGCTCAGGA TGTTCGAGGA
2901  CAGGTGTCCT TTTATCGGGG TTCACTTCCT GTGGAGGTAC CTGCAGAGGC
2951  AGAGGCTGCC CGGCAGAGGA AGGGTACAGA TGCACTGTGG ATGGCCACCT
3001  TGCCCATCAA GCTACCGAGA CTCCGGGGTG CCCAGGGGAG TGGGCAGGGG
3051  ACTGACTTCT CCTTGATGCC TCTGAACCTG GGTGATGCAG AAACTGGATT
3101  CCTGACTCAG AGCAACCTGC TGAGTGTGGC CTCACGCCTA GGTCCTGACT
3151  GGCCAGCTGT GGCCCTGCAC CTAGGCATGC CCTACCATAA GCTACAGCGT
3201  ATCCGGCATG AATTCAGGGA TGACCTGGAT GGCCAGGTCC GACACATGCT
3251  CTTCTCTTGG GCTGAGCGCC AGACTGGACA GCCTGGAGCT GTGGGACACC
3301  TGGTACAGGC CTTGGAACAG AGTGATCGGC GGGATGTGGC TGAAGAGGTG
3351  CGTGCCATCT TGGAGCTTGG CCGCCACAAG TACCAGGACA GCATTCGACG
3401  CACAGGGCTG GCCCCTGAGG ATTCTACTCT GCCTGGCACC TCGGCTTCAC
3451  AGACTCCAGA GTCTGCACAG GCCTAGGCTT ACAGACTTAA TTAAGATAGC  STOP
3501  TTTAAACTTG ACTCGACCAG TGGGCAGAGC CCTATCCTTC TACCTCACCC
3551  GTGTGCTCCA GTGCCCCAGC ATACAAAAAC CCACTCACTG TCTAGCCCAA
3601  GCTAGCCTCC AACTCACAGT GAGTACATCT CAGAGATTGT TTTACATAAA
3651  GGATAGAGAG CAGGTTGCCC ATACTTGCTA CCATGCCAGG CCCTTCTGCT
3701  AAGATGGGAG CAGAGAAGGC CTGGAGGGAG AGATGGCTCA GTAGTTAACA
3751  ACACTAGCTG CTCCTTCAGG GAACCTAGTT TGTTTCCTCT ACCTACCTGA
3801  TGGCTCATGA CAATCTGTAA CTCCAGTTCT GAAGGGTCAG CCCCTTCCTC
3851  TGGCCCCCAC AGACACCAGG CACACATGTG TGCAGACAAG TATGCAGGCA
3901  AAACATCCAT ACAAATAAAA TAAAAGCTAT TTATTGTG
```

FIGURE 6

hPIDD Length: 1943 September 2, 1999 16:49 Type: N Check: 4760 ..

1 GAAACAGCTG CTCTGGGAGG CGGCAGCAGG AGTGTCCCAG CGCCGTGGGC

51 TGGACTGGTG CCAAGCCTAA GCCAGCACCT GCCCGCAGCG GGACTTCGGT

101 CCTTGCGGCT CCTTGTCCTG CACAGCAACC TCCTGGCCTC TGTGCCAGCT

151 GACTTGGCCC GCCTTCCACT CCTCACCCGG CTCGACCTGA GGGACAACCA

201 GCTCCGGGAC CTGCCCCCTG AGCTGCTAGA CGCCCCCTTT GTGCGCCTGC

251 AGGGGAACCC CCTGGGTGAG GCCTCGCCAG ACGCCCCGAG TTCACCAGTG

301 GCAGCCCTCA TTCCAGAAAT GCCCAGACTG TTCCTGACCT CAGATTTGGA

351 CAGCTTTCCT GTGACCCCTC GAGGCTGCTC AGTGACCCTG GCCTGTGGCG

401 TCCGCCTGCA GTTCCCAGCG GGAGCCACCG CCACCCCCAT CACCATCCGC

451 TATCGGCTGC TGCTGCCGGA GCCAGGCCTC GTCCCCCTGG GTCCTCATGA

501 CGCCCTGCTC AGCCATGTGC TGGAGCTGCA GCCCCATGGG GTGGCCTTCC

551 AGCAGGATGT GGGGCTGTGG CTGCTCTTCA CCCCACCGCA GGCCCGGCGC

601 TGCCGTGAAG TGGTGGTCAG GACCCGGAAT GACAACAGCT GGGGTGACCT

651 GGAGACCTAC CTGGAGGAAG AGGCACCCCA GCGGCTCTGG GCTCACTGCC

701 AGGTGCCCCA CTTCTCCTGG TTCCTTGTGG TTTCCCGCCC TGTGTCCAAT

751 GCCTGCTTGG TGCCACCGGA GGGGACACTG CTGTGCTCCT CGGGTCATCC

801 TGGGGTCAAA GTCATCTTCC CCCCTGGGGC CACTGAGGAG CCTCGTCGAG

851 TCTCCATGCA GGTGGTGCGC ATGGCTGGCC GAGAGCTGCA GGCCCTCCTG

901 GGAGAACCAG AGGCTGCAGT GAGCCCCCTG CTGTGCCTGT CACAGAGCGG

FIGURE 6 (CONT'D)

951 TCCCCCCAGC TTCCTCCAAC CGGTCACCGT GCAGCTGCCT CTGCCCTCTG

1001 GCATCACAGG CCTCAGTCTG GACCGCTCCC GCCTGCACCT GTTGTACTGG

1051 GCCCCTCCTG CAGCCACCTG GGATGACATC ACAGCTCAGG TGGTCCTGGA

1101 GCTCACCCAC CTGTACGCAC GCTTCCAGGT CACACACTTC TCCTGGTACT

1151 GGTTCTGGTA CACCACCAAG AACTGTGTGG GAGGCCTGGC TCGGAAGGCC

1201 TGGGAGCGGC TGCGGCTGCA CCGTGTGAAC CTCATCGCTC TGCAGCGGCG

1251 CCGGGACCCT GAGCAGGTCC TGCTGCAGTG CCTGCCCCGA AACAAGGTGG

1301 ACGCCACCCT TCGGCGGCTG CTGGAGCGGT ACCGGGGCCC CGA[.....]$_n$* GC

1351 TCAGGCTGTG CGGGGCCAGG TGTCCTTCTA CCGTGGCGCG GTGCCTGTGC

1401 GGGTGCCCGA GGAGGCTGAG GCTGCCCGGC AGAGGAAGGG CGCAGACGCC

1451 CTGTGGATGG CCACTCTGCC CATCAAGCTG CCGAGACTTC GAGGGTCCGA

1501 GGGGCCACGG CGGGGGGCTG GCCTCTCCTT GGCACCCTTG AATCTGGGAG

1551 ATGCCGAGAC CGGCTTTCTG ACGCAGAGCA ACCTGCTGAG TGTGGCTGGG

1601 CGTCTGGGTC TGGACCGGCC AGCCGTGGCC CTGCACCTGG GGGTGTCCTA

1651 CCGGGAGGTG CAGCGCATCC GGCACGAGTT CCGGGATGAT CTGGATGAGC

1701 AGATCCGTCA CATGCTCTTC TCCTGGGCTG AGCGCCAGGC TGGGCAGCCA

1751 GGGGCTGTGG GGCTCCTGGT GCAGGCCCTG GAGCAGAGTG ACCGGCAGGA

1801 CGTGGCTGAA GAGGTGCGCG CAGTCTTGGA GCTCGGCCGC CGCAAGTACC

1851 AGGACAGCAT CCGACGCATG GGCTTGGCCC CCAAGGACCC CGCTCTGCCT

1901 GGCTCCTCGG CTCCACAGCC CCCAGAGCCT GCCCGAGCCT AGGC

[.....]$_n$ refers to 175 base DNA sequence between nucleotides 2107 and 2283.

FIGURE 7

cDNA sequence identity (%) between the death domain of Pidd and that of other death domain-containing proteins

	hPIDD	hFADD	hRIP	hRAIDD	hDAPK
mPidd	81	44.2	44.4	45.8	45.8
hPIDD		45.4	46.1	44.9	43.3

FIGURE 8

NCBI **Blast 2 Sequences results**

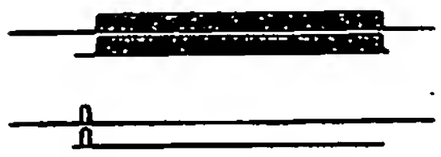
PubMed Entrez BLAST OMIM Taxonomy Structure

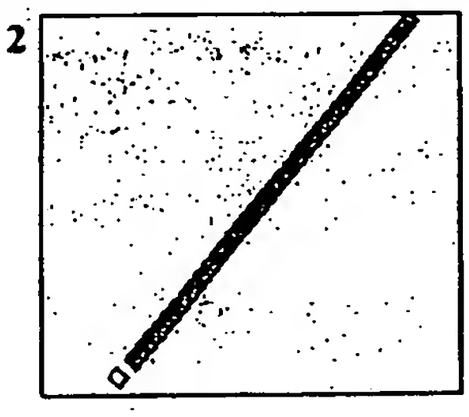
BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.1.2 [Oct-19-2000]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2
x_dropoff: 50 expect: 10.00000 wordsize: 11 Filter Align

Sequence 1 lcl|seq_1 **Length** 3938 (1 .. 3938) **Mouse Pidd (refer to Fig 5)**

Sequence 2 lcl|seq_2 **Length** 2900 (1 .. 2900) **Human PIDD (Fig 10)**





NOTE:The statistics (bitscore and expect value) is calculated based on the size of nr database

```
Score = 2305 bits (1199), Expect = 0.0
Identities = 2153/2630 (81%)
 Strand = Plus / Plus
```

```
Mouse    Query: 849   aaccagttgaacttggacctacgcccaggggctgccatcggctacagtacctgtgttcc 908
                      ||||  |  |||  |||||||||    |||  ||||||||||||  |  |||  |  |  |||||||||   |
Human    Sbjct: 249   aaccggctgagcttggacctgtaccccgggggctgccagcagctgctgcacctgtgtgtc 308

Query: 909   cagcagcccccacagctgctgcaagtggagtttttgcggctcagcacccatgaggaccct 968
              ||||||||  |  ||||||||||| |||||  ||  |||||  ||  |||||  ||  |||||||||
Sbjct: 309   cagcagcctcttcagctgctgcaggtggaattcttgcgtctgagcactcacgaggaccct 368

Query: 969   cagctgctggatgacaccctagccaaggttccgtggagtctgctgcgccttcgctccctg 1028
              ||||||||||| | ||||||  |||  ||  |  ||   |||  |||    ||||  |||||||||
Sbjct: 369   cagctgctggaggccaccctggcccagctgcctcagagcctgtcctgcctccgctccctg 428

Query: 1029  gtcctcaaaggtggccagagccggggtgccctgggtgcctgcctccatggaaccctgacc 1088
              ||||||||||  ||  ||  ||||||   |  ||||||||||  ||||  ||  ||||||||
Sbjct: 429   gtcctcaaaggagggcaacgccgggacacactgggtgcctgtctccggggtgccctgacc 488

Query: 1089  actctgcctgctggcctgagtgatttggcctgcctggctcacctagacctgagcttcaac 1148
              |  |||||  |||||  |||||||   |||||   |||||  |||||  ||||||||||||||
Sbjct: 489   aacctgcccgctggtctgagtggcctggcccatctggcccacctggacctgagcttcaac 548
```

15/23
FIGURE 8 (CONT'D)

```
Query: 1149 aggctggagacgctgcctacctgtgtcccggaactgcacggcctggatgcgttgctgctt 1208
            || |||||||| |||||  |||||||| | |  |||  || |||| |||| |  ||||
Sbjct: 549  agcctggagacactgccggcctgtgtcctgcagatgcgaggtctgggtgcgctcttgctg 608

Query: 1209 tctcacaaccatctctcagagctgcccgaggccctgggggcactgcccgcccttaccttc 1268
            |||||||||   |||||  |||||||  |||||  |||||||  ||  |||||||| ||||||
Sbjct: 609  tctcacaactgcctctctgagctgcctgaggctctgggggccctccccgccctcaccttc 668

Query: 1269 ctcaccgtgacacacaaccgcctagaaagactacccctaacactcgggtccctgtccacc 1328
            |||||  |||||||||||||||||  | |  ||  ||||  |  ||||  |||  ||||  ||||||
Sbjct: 669  ctcacagtgacacacaaccgcctgcagacgctgcccccagcactgggggccctatccacc 728

Query: 1329 cttcagcgtctcgatctctctgagaaccttctagacaccataccctctgagattggaaac 1388
            ||  |||||  ||||||||||||  ||||  ||  ||  |||||   ||||   ||||||||||   |
Sbjct: 729  ctgcagcgcctcgatctctctcagaatctgctggacacgctacctcctgagattggaggc 788

Query: 1389 ctgcgcagcctcagtgagctcaatctggcctccaaccggctgcagagtctcccagcttcc 1448
            |||  ||||||||   |||||||  ||||||||||||||||||||||||  ||||||||  ||
Sbjct: 789  ctgggcagcctcctggagctcaacctggcctccaaccggctgcagagcctcccagcctct 848

Query: 1449 ctcgcggggctgcggtccctgcggctccttgttctgcacagcaacctcctgacctcagtg 1508
            ||  |||||  ||  ||||||  ||||||||||||  ||||||||||||||||||  ||||  |||
Sbjct: 849  ctggcgggacttcggtccttgcggctccttgtcctgcacagcaacctcctggcctctgtg 908

Query: 1509 cccactggcctggtccacctgccactgatcactcggcttgacctgagggacaaccggctc 1568
            ||  |||  |  |||  ||  |||  |||||  ||||  |||||  ||||||||||||||  ||||
Sbjct: 909  ccagctgacttggcccgccttccactcctcacccggctcgacctgagggacaaccagctc 968

Query: 1569 cgagacctgcctgctgagttactagacgctccctttgtgcgcctgcaggggaaccctctg 1628
            ||  |||||||||  |||||  |  ||||||||  ||||||||||||||||||||||||||  |||
Sbjct: 969  cgggacctgccccctgagctgctagacgcccccctttgtgcgcctgcaggggaacccccctg 1028

Query: 1629 ggcgaggcctctccagcacccccaagtcccccagacatatcccaggttccagaaatgccc 1688
            ||  |||||||||  ||||   ||||  |||  |  ||||    |  |||   ||||||||||||||
Sbjct: 1029 ggtgaggcctcgccagacgccccgagttcaccagtggcagccctcattccagaaatgccc 1088

Query: 1689 aggctattgctgacctcagatttggacagcttccttgtgactccccatggctgttctgtg 1748
            ||  ||  ||  |||||||||||||||||||||||  |  ||||||  ||  |  |||||  ||  |||
Sbjct: 1089 agactgttcctgacctcagatttggacagctttcctgtgacccctcgaggctgctcagtg 1148

Query: 1749 accctggcctgtggtgtccgcctacagtttccagcaggagccaccaccacgcctgtcacc 1808
            ||||||||||||||  ||||||||  |||||  |||||  |||||||||  ||||  ||  |||||
Sbjct: 1149 accctggcctgtggcgtccgcctgcagttcccagcgggagccaccgccacccccatcacc 1208
```

FIGURE 8 (CONT'D)

```
Query: 1809 atccactatcgactctggttgcccgagccaggcctggtctctctgggacctcatgacttc 1868
           |||| |||||| ||   | |||| |||||||||||| ||| | ||||| |||||||||  |
Sbjct: 1209 atccgctatcggctgctgctgccggagccaggcctcgtccccctgggtcctcatgacgcc 1268

Query: 1869 ctgcttagtagtgtcctggagctgcagccccatggggtggctttccagcaagatgtgagc 1928
           |||||  ||   ||| |||||||||||||||||||||||||| |||||||| |||||| |
Sbjct: 1269 ctgctcagccatgtgctggagctgcagccccatggggtggccttccagcaggatgtgggg 1328

Query: 1929 ttatggctgctctttgtccctccacgagtccgtcgctgtcgtgaggtagttgtgaggaca 1988
           | |||||||||||   ||| || |  | ||| ||||| ||||| || || || |||||
Sbjct: 1329 ctgtggctgctcttcaccccaccgcaggcccggcgctgccgtgaagtggtggtcaggacc 1388

Query: 1989 cggagtaacaacacctggaatgacctagagacccaactggaggaagaggcacccaagagg 2048
           |||| | |||||| ||||  |||||| |||||| | ||||||||||||||||| || ||
Sbjct: 1389 cggaatgacaacagctggggtgacctggagacctacctggaggaagaggcaccccagcgg 1448

Query: 2049 ctctgggctcgctgccaggtgccccacttctcctggttcctggtcgttttacgcccagta 2108
           |||||||||| |||||||||||||||||||||||||||||| || ||||  ||||| ||
Sbjct: 1449 ctctgggctcactgccaggtgccccacttctcctggttccttgtggtttcccgccctgtg 1508

Query: 2109 tccaacacttgccttttgccaccagagggagcacttctctgctcctcaggtcatcctggg 2168
           |||||  | |||||  ||||||| ||||| |||| || |||||||| ||||||||||||
Sbjct: 1509 tccaatgcctgcctggtgccaccggaggggacactgctgtgctcctcgggtcatcctggg 1568

Query: 2169 gtcagagtcacttttccccctggggttacagaagagcctcggcaagtctccatgcaggta 2228
           |||| |||||  || |||||||||  || || |||||||| | ||||||||||||||
Sbjct: 1569 gtcaaagtcatcttcccccctggggccactgaggagcctcgtcgagtctccatgcaggtg 1628

Query: 2229 gtgcatatggctggactagagctgagaacccttctggaagaatcagaggcatcagtaagc 2288
           ||||  |||||||| | |||||||    |||| |||| |||| |||||||  |||| |||
Sbjct: 1629 gtgcgcatggctggccgagagctgcaggccctcctgggagaaccagaggctgcagtgagc 1688

Query: 2289 cctctgctgtgcctttcacagagcggccccccagcttcctacaacctgtcactgtgcag 2348
           || |||||||||| |||||||||| |||||||||||||| ||||| ||||| ||||||
Sbjct: 1689 cccctgctgtgcctgtcacagagcggtcccccagcttcctccaaccggtcaccgtgcag 1748

Query: 2349 ttgcccctgcctcctggcgttacaggcttcagtctagaccgctcccacctgcatctgctc 2408
           |||| |||||  ||||| | |||||| ||||||| |||||||||| |||||| ||| |
Sbjct: 1749 ctgcctctgccctctggcatcacaggcctcagtctggaccgctcccgcctgcacctgttg 1808

Query: 2409 taccgaacacccctgacaaccacctgggatgatatcaccactcaggtggcactggaattc 2468
           ||| |  | || |   || |||||||||||| |||||  |||||||||  |||||  ||
Sbjct: 1809 tactgggcccctcctgcagccacctgggatgacatcacagctcaggtggtcctggagctc 1868
```

FIGURE 8 (CONT'D)

```
Query: 2469 acccacctgtatgcacgcttccaggtcacgcacttctcctggtactggctctggtatacc 2528
Sbjct: 1869 acccacctgtacgcacgcttccaggtcacacacttctcctggtactggctctggtacacc 1928

Query: 2529 accaaaacctgtgtgggggcctggcccggaaggcctgggaacggctgcgactgcaccgt 2588
Sbjct: 1929 accaagaactgtgtgggaggcctggctcggaaggcctgggagcggctgcggctgcaccgt 1988

Query: 2589 gtgaatctcattgcactgcagaggcgccgagaccctgagcaggttctgctgcagtgcctg 2648
Sbjct: 1989 gtgaacctcatcgctctgcagcggcgccgggaccctgagcaggtcctgctgcagtgcctg 2048

Query: 2649 ccacggaacaaggtagatgccaccctgagtcggctgctggttcgctaccgtggccctgaa 2708
Sbjct: 2049 ccccgaaacaaggtggacgccacccttcggcggctgctggagcggtaccggggccccgag 2108

Query: 2709 ccctctgagactgtggagatgtttgagggtgagaagttctttgcagcctttgagcggggc 2768
Sbjct: 2109 ccctctgacacggtggagatgttcgagggcgaagagttctttgcggccttcgagcgcggc 2168

Query: 2769 attgatgtagatgcagaccgtccagactgtgtggatggcagaatctgctttgtcttctat 2828
Sbjct: 2169 atcgacgtggatgctgaccgccctgactgtgtggagggcagaatctgctttgtcttctac 2228

Query: 2829 tcacacctgaagaacgtaaaggaggtatacatcaccacagccttggaccgggaagctcag 2888
Sbjct: 2229 tcgcacctgaagaatgtgaaggaggtatacgtgaccaccactctggaccgggaggctcag 2288

Query: 2889 gatgttcgaggacaggtgtccttttatcggggttcacttcctgtggaggtacctgcagag 2948
Sbjct: 2289 gctgtgcggggccaggtgtccttctaccgtggcgcggtgcctgtgcgggtgcccgaggag 2348

Query: 2949 gcagaggctgcccggcagaggaagggtacagatgcactgtggatggccaccttgcccatc 3008
Sbjct: 2349 gctgaggctgcccggcagaggaagggcgcagacgccctgtggatggccactctgcccatc 2408

Query: 3009 aagctaccgagactccggggtgcccaggggagtgggcaggggactgacttctccttgatg 3068
Sbjct: 2409 aagctgccgagacttcgagggtccgaggggccacggcgggggctggcctctccttggca 2468

Query: 3069 cctctgaacctgggtgatgcagaaactggattcctgactcagagcaacctgctgagtgtg 3128
Sbjct: 2469 cccttgaatctgggagatgccgagaccggctttctgacgcagagcaacctgctgagtgtg 2528

Query: 3129 gcctcacgcctaggtcctgactggccagctgtggccctgcacctaggcatgccctaccat 3188
Sbjct: 2529 gctgggcgtctgggtctggactggccagccgtggccctgcacctgggggtgtcctaccgg 2588

Query: 3189 aagctacagcgtatccggcatgaattcagggatgacctggatggccaggtccgacacatg 3248
Sbjct: 2589 gaggtgcagcgcatccggcacgagttccgggatgatctggatgagcagatccgtcacatg 2648
```

FIGURE 8 (CONT'D)

```
Query: 3249 ctcttctcttgggctgagcgccagactggacagcctggagctgtgggacacctggtacag 3308
            |||||||| |||||||||||||||| |||| ||||| || |||||||| | |||||| |||
Sbjct: 2649 ctcttctcctgggctgagcgccaggctgggcagccaggggctgtggggctcctggtgcag 2708

Query: 3309 gccttggaacagagtgatcggcgggatgtggctgaagaggtgcgtgccatcttggagctt 3368
            ||| |||| |||||||| |||| ||| |||||||||||||||| ||  ||||||||||
Sbjct: 2709 gccctggagcagagtgaccggcaggacgtggctgaagaggtgcgcgcagtcttggagctc 2768

Query: 3369 ggccgccacaagtaccaggacagcattcgacgcacagggctggcccctgaggattctact 3428
            ||||||| |||||||||||||||||| ||||||| ||  ||||||| ||||  |  ||
Sbjct: 2769 ggccgccgcaagtaccaggacagcatccgacgcatgggcttggcccccaaggaccccgct 2828

Query: 3429 ctgcctggcacctcggcttcacagactccagagtctgcacaggcctaggc 3478
            ||||||||| |||||||| ||||| | |||||| |||| ||||||||||
Sbjct: 2829 ctgcctggctcctcggctccacagcccccagagcctgcccaggcctaggc 2878

Score = 68.0 bits (35), Expect = 2e-08
Identities = 65/80 (81%)
 Strand = Plus / Plus
```

```
Mouse   Query: 690 gctgggacatgtctggtccccgaggaccttccatgggtgatggctgcagtgttggagggg 749
                   |||||||||||||||| | || |||||| ||  |||| ||||||||||  | ||||||||
Human   Sbjct: 105 gctgggacatgtctggcctccaaggaccgtcggtgggcgatggctgcaacggtggagggg 164

Query: 750 caggagccggaggaaactgc 769
           |  |||| ||||| | ||||
Sbjct: 165 ccagagctggaggcagctgc 184

CPU time:      0.19 user secs.      0.02 sys. secs          0.21 total secs.

Gapped
Lambda      K       H
    1.33     0.621      1.12

Gapped
Lambda      K       H
    1.33     0.621      1.12

Matrix: blastn matrix:1 -2
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 29
Number of Sequences: 0
Number of extensions: 29
Number of successful extensions: 9
Number of sequences better than 10.0: 1
length of query: 2900
length of database: 2,484,783,180
effective HSP length: 25
effective length of query: 2875
effective length of database: 2,484,783,155
effective search space: 7143751570625
effective search space used: 7143751570625
T: 0
A: 0
X1: 6 (11.5 bits)
X2: 26 (50.0 bits)
S1: 12 (23.8 bits)
S2: 21 (41.1 bits)
```

FIGURE 9

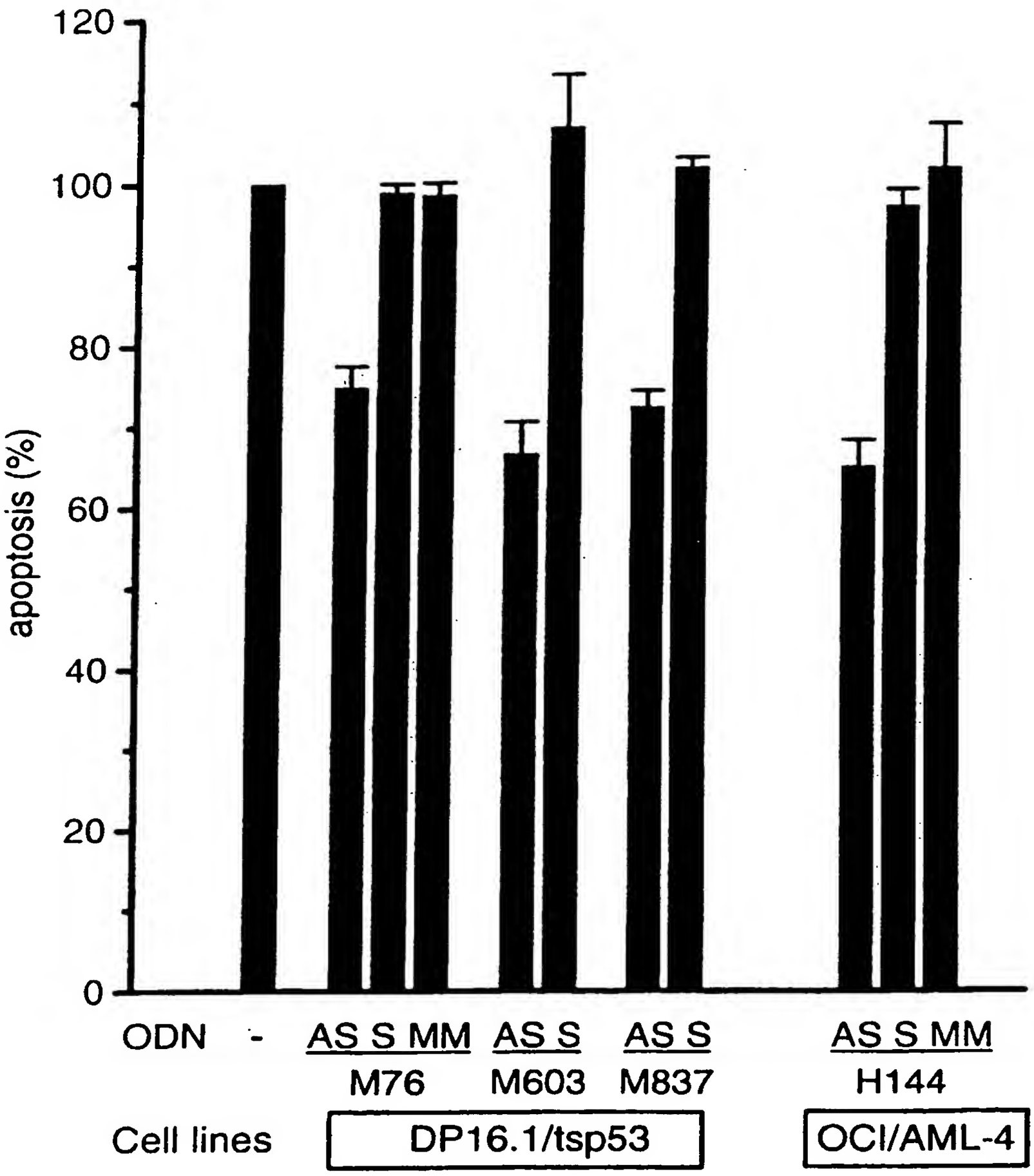

120
100
80
60
40
20
0
apoptosis (%)
ODN - AS S MM AS S AS S AS S MM
M76 M603 M837 H144
Cell lines DP16.1/tsp53 OCI/AML-4

FIGURE 10

Human PIDD cDNA sequence

```
   1  GGCCGGCGGG ATTCCAGGCG CCGAGCGCCC GCTGAGCAGC CACCCTTTGC
  51  GCGCCGCCTG CAGCGCAGCT TCCCCGGGCG CTGCCTGGAC AGGCCTGCCT
 101  GCGTGCTGGG ACATGTCTGG CCTCCAAGGA CCGTCGGTGG GCGATGGCTG  Met1
 151  CAACGGTGGA GGGGCCAGAG CTGGAGGCAG CTGCTGCCGC AGGAGATGCT
 201  TCAGAGGATT CGGACGCAGG GTCCAGGGCG CTGCCTTTCC TGGGCGGCAA
 251  CCGGCTGAGC TTGGACCTGT ACCCCGGGGG CTGCCAGCAG CTGCTGCACC
 301  TGTGTGTCCA GCAGCCTCTT CAGCTGCTGC AGGTGGAATT CTTGCGTCTG
 351  AGCACTCACG AGGACCCTCA GCTGCTGGAG GCCACCCTGG CCCAGCTGCC
 401  TCAGAGCCTG TCCTGCCTCC GCTCCCTGGT CCTCAAAGGA GGGCAACGCC
 451  GGGACACACT GGGTGCCTGT CTCCGGGGTG CCCTGACCAA CCTGCCCGCT
 501  GGTCTGAGTG GCCTGGCCCA TCTGGCCCAC CTGGACCTGA GCTTCAACAG
 551  CCTGGAGACA CTGCCGGCCT GTGTCCTGCA GATGCGAGGT CTGGGTGCGC
 601  TCTTGCTGTC TCACAACTGC CTCTCTGAGC TGCCTGAGGC TCTGGGGGCC
 651  CTCCCCGCCC TCACCTTCCT CACAGTGACA CACAACCGCC TGCAGACGCT
 701  GCCCCCAGCA CTGGGGGCCC TATCCACCCT GCAGCGCCTC GATCTCTCTC
 751  AGAATCTGCT GGACACGCTA CCTCCTGAGA TTGGAGGCCT GGGCAGCCTC
 801  CTGGAGCTCA ACCTGGCCTC CAACCGGCTG CAGAGCCTCC CAGCCTCTCT
 851  GGCGGGACTT CGGTCCTTGC GGCTCCTTGT CCTGCACAGC AACCTCCTGG
 901  CCTCTGTGCC AGCTGACTTG GCCCGCCTTC CACTCCTCAC CCGGCTCGAC
 951  CTGAGGGACA ACCAGCTCCG GGACCTGCCC CCTGAGCTGC TAGACGCCCC
1001  CTTTGTGCGC CTGCAGGGGA ACCCCCTGGG TGAGGCCTCG CCAGACGCCC
1051  CGAGTTCACC AGTGGCAGCC CTCATTCCAG AAATGCCCAG ACTGTTCCTG
1101  ACCTCAGATT TGGACAGCTT TCCTGTGACC CCTCGAGGCT GCTCAGTGAC
1151  CCTGGCCTGT GGCGTCCGCC TGCAGTTCCC AGCGGGAGCC ACCGCCACCC
1201  CCATCACCAT CCGCTATCGG CTGCTGCTGC CGGAGCCAGG CCTCGTCCCC
1251  CTGGGTCCTC ATGACGCCCT GCTCAGCCAT GTGCTGGAGC TGCAGCCCCA
```

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FIGURE 10 (CONT'D)

1301 TGGGGTGGCC TTCCAGCAGG ATGTGGGGCT GTGGCTGCTC TTCACCCCAC

1351 CGCAGGCCCG GCGCTGCCGT GAAGTGGTGG TCAGGACCCG GAATGACAAC

1401 AGCTGGGGTG ACCTGGAGAC CTACCTGGAG GAAGAGGCAC CCCAGCGGCT

1451 CTGGGCTCAC TGCCAGGTGC CCCACTTCTC CTGGTTCCTT GTGGTTTCCC

1501 GCCCTGTGTC CAATGCCTGC CTGGTGCCAC CGGAGGGGAC ACTGCTGTGC

1551 TCCTCGGGTC ATCCTGGGGT CAAAGTCATC TTCCCCCCTG GGGCCACTGA

1601 GGAGCCTCGT CGAGTCTCCA TGCAGGTGGT GCGCATGGCT GGCCGAGAGC

1651 TGCAGGCCCT CCTGGGAGAA CCAGAGGCTG CAGTGAGCCC CCTGCTGTGC

1701 CTGTCACAGA GCGGTCCCCC CAGCTTCCTC CAACCGGTCA CCGTGCAGCT

1751 GCCTCTGCCC TCTGGCATCA CAGGCCTCAG TCTGGACCGC TCCCGCCTGC

1801 ACCTGTTGTA CTGGGCCCCT CCTGCAGCCA CCTGGGATGA CATCACAGCT

1851 CAGGTGGTCC TGGAGCTCAC CCACCTGTAC GCACGCTTCC AGGTCACACA

1901 CTTCTCCTGG TACTGGCTCT GGTACACCAC CAAGAACTGT GTGGGAGGCC

1951 TGGCTCGGAA GGCCTGGGAG CGGCTGCGGC TGCACCGTGT GAACCTCATC

2001 GCTCTGCAGC GGCGCCGGGA CCCTGAGCAG GTCCTGCTGC AGTGCCTGCC

2051 CCGAAACAAG GTGGACGCCA CCCTTCGGCG GCTGCTGGAG CGGTACCGGG

2101 GCCCCGAGCC CTCTGACACG GTGGAGATGT TCGAGGGCGA AGAGTTCTTT

2151 GCGGCCTTCG AGCGCGGCAT CGACGTGGAT GCTGACCGCC CTGACTGTGT

2201 GGAGGGCAGA ATCTGCTTTG TCTTCTACTC GCACCTGAAG AATGTGAAGG

2251 AGGTATACGT GACCACCACT CTGGACCGGG AGGCTCAGGC TGTGCGGGGC

2301 CAGGTGTCCT TCTACCGTGG CGCGGTGCCT GTGCGGGTGC CCGAGGAGGC

2351 TGAGGCTGCC CGGCAGAGGA AGGGCGCAGA CGCCCTGTGG ATGGCCACTC

2401 TGCCCATCAA GCTGCCGAGA CTTCGAGGGT CCGAGGGGCC ACGGCGGGGG

2451 GCTGGCCTCT CCTTGGCACC CTTGAATCTG GGAGATGCCG AGACCGGCTT

2501 TCTGACGCAG AGCAACCTGC TGAGTGTGGC TGGGCGTCTG GGTCTGGACT

2551 GGCCAGCCGT GGCCCTGCAC CTGGGGGTGT CCTACCGGGA GGTGCAGCGC

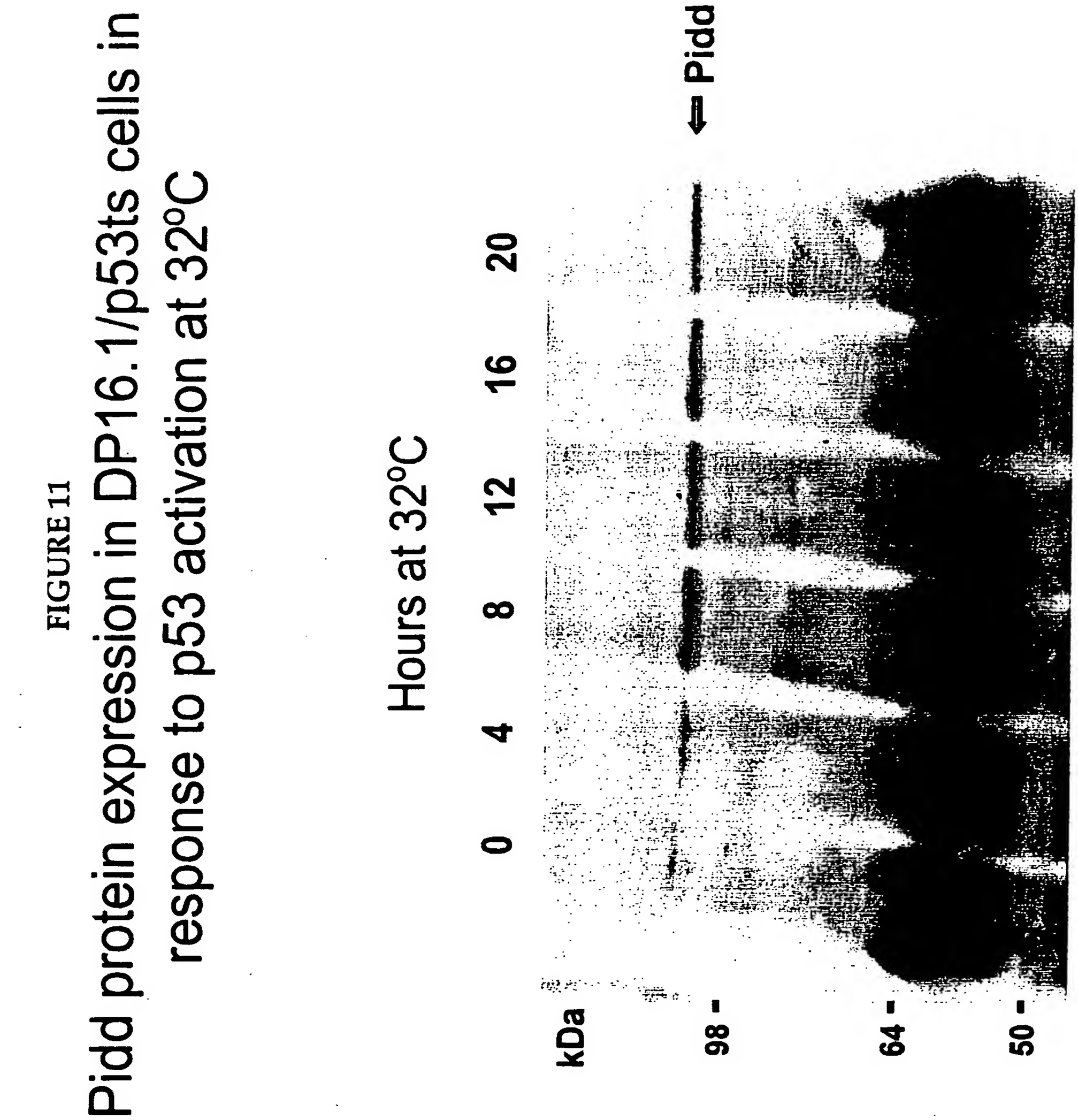
2601 ATCCGGCACG AGTTCCGGGA TGATCTGGAT GAGCAGATCC GTCACATGCT

FIGURE 10 (CONT'D)

```
2651  CTTCTCCTGG GCTGAGCGCC AGGCTGGGCA GCCAGGGGCT GTGGGGCTCC
2701  TGGTGCAGGC CCTGGAGCAG AGTGACCGGC AGGACGTGGC TGAAGAGGTG
2751  CGCGCAGTCT TGGAGCTCGG CCGCCGCAAG TACCAGGACA GCATCCGACG
2801  CATGGGCTTG GCCCCCAAGG ACCCCGCTCT GCCTGGCTCC TCGGCTCCAC
2851  AGCCCCCAGA GCCTGCCCAG GCCTAGGCCC CACAGACTTT TAGGCTGGCC  STOP
```

FIGURE 11

Pidd protein expression in DP16.1/p53ts cells in response to p53 activation at 32°C



SEQUENCE LISTING

<110> Benchimol, Samuel
Lin, Yunping
University Health Network

<120> A P53-Induced Protein with a Death Domain that Can Promote Apoptosis

<130> 10723-17

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<150> 60/152,359
<151> 1999-09-07

<150> 60/211,086
<151> 2000-06-13

<160> 31

<170> PatentIn Ver. 2.0

<210> 1
<211> 915
<212> PRT
<213> Murine

<400> 1

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Met Ala Ala Val Leu Glu Gly Gln Glu Pro Glu Glu Thr Ala Ala Ala
  1               5                  10                  15

Ala Glu Asp Ala Ala Thr Ser Thr Leu Glu Ala Val Asp Ala Gly Pro
             20                  25                  30

Gly Ala Pro Phe Leu Pro Ala Gly Asn Gln Leu Asn Leu Asp Leu Arg
         35                  40                  45

Pro Gly Gly Cys His Arg Leu Gln Tyr Leu Cys Ser Gln Gln Pro Pro
     50                  55                  60

Gln Leu Leu Gln Val Glu Phe Leu Arg Leu Ser Thr His Glu Asp Pro
 65                  70                  75                  80

Gln Leu Leu Asp Asp Thr Leu Ala Lys Val Pro Trp Ser Leu Leu Arg
                 85                  90                  95

Leu Arg Ser Leu Val Leu Lys Gly Gly Gln Ser Arg Gly Ala Leu Gly
            100                 105                 110

Ala Cys Leu His Gly Thr Leu Thr Thr Leu Pro Ala Gly Leu Ser Asp
        115                 120                 125

Leu Ala Cys Leu Ala His Leu Asp Leu Ser Phe Asn Arg Leu Glu Thr
    130                 135                 140

Leu Pro Thr Cys Val Pro Glu Leu His Gly Leu Asp Ala Leu Leu Leu
145                 150                 155                 160

Ser His Asn His Leu Ser Glu Leu Pro Glu Ala Leu Gly Ala Leu Pro
                165                 170                 175

Ala Leu Thr Phe Leu Thr Val Thr His Asn Arg Leu Glu Arg Leu Pro
            180                 185                 190
```

Leu Thr Leu Gly Ser Leu Ser Thr Leu Gln Arg Leu Asp Leu Ser Glu
195 200 205

Asn Leu Leu Asp Thr Ile Pro Ser Glu Ile Gly Asn Leu Arg Ser Leu
210 215 220

Ser Glu Leu Asn Leu Ala Ser Asn Arg Leu Gln Ser Leu Pro Ala Ser
225 230 235 240

Leu Ala Gly Leu Arg Ser Leu Arg Leu Leu Val Leu His Ser Asn Leu
245 250 255

Leu Thr Ser Val Pro Thr Gly Leu Val His Leu Pro Leu Ile Thr Arg
260 265 270

Leu Asp Leu Arg Asp Asn Arg Leu Arg Asp Leu Pro Ala Glu Leu Leu
275 280 285

Asp Ala Pro Phe Val Arg Leu Gln Gly Asn Pro Leu Gly Glu Ala Ser
290 295 300

Pro Ala Pro Pro Ser Pro Pro Asp Ile Ser Gln Val Pro Glu Met Pro
305 310 315 320

Arg Leu Leu Leu Thr Ser Asp Leu Asp Ser Phe Leu Val Thr Pro His
325 330 335

Gly Cys Ser Val Thr Leu Ala Cys Gly Val Arg Leu Gln Phe Pro Ala
340 345 350

Gly Ala Thr Thr Thr Pro Val Thr Ile His Tyr Arg Leu Trp Leu Pro
355 360 365

Glu Pro Gly Leu Val Ser Leu Gly Pro His Asp Phe Leu Leu Ser Ser
370 375 380

Val Leu Glu Leu Gln Pro His Gly Val Ala Phe Gln Gln Asp Val Ser
385 390 395 400

Leu Trp Leu Leu Phe Val Pro Pro Arg Val Arg Arg Cys Arg Glu Val
405 410 415

Val Val Arg Thr Arg Ser Asn Asn Thr Trp Asn Asp Leu Glu Thr Gln
420 425 430

Leu Glu Glu Glu Ala Pro Lys Arg Leu Trp Ala Arg Cys Gln Val Pro
435 440 445

His Phe Ser Trp Phe Leu Val Val Leu Arg Pro Val Ser Asn Thr Cys
450 455 460

Leu Leu Pro Pro Glu Gly Ala Leu Leu Cys Ser Ser Gly His Pro Gly
465 470 475 480

Val Arg Val Thr Phe Pro Pro Gly Val Thr Glu Glu Pro Arg Gln Val
485 490 495

Ser Met Gln Val Val His Met Ala Gly Leu Glu Leu Arg Thr Leu Leu
500 505 510

Glu Glu Ser Glu Ala Ser Val Ser Pro Leu Leu Cys Leu Ser Gln Ser
515 520 525

Gly Pro Pro Ser Phe Leu Gln Pro Val Thr Val Gln Leu Pro Leu Pro
530 535 540

Pro Gly Val Thr Gly Phe Ser Leu Asp Arg Ser His Leu His Leu Leu
545 550 555 560

Tyr Arg Thr Pro Leu Thr Thr Thr Trp Asp Asp Ile Thr Thr Gln Val
565 570 575

Ala Leu Glu Phe Thr His Leu Tyr Ala Arg Phe Gln Val Thr His Phe
580 585 590

Ser Trp Tyr Trp Leu Trp Tyr Thr Thr Lys Thr Cys Val Gly Gly Leu
595 600 605

Ala Arg Lys Ala Trp Glu Arg Leu Arg Leu His Arg Val Asn Leu Ile
610 615 620

Ala Leu Gln Arg Arg Arg Asp Pro Glu Gln Val Leu Leu Gln Cys Leu
625 630 635 640

Pro Arg Asn Lys Val Asp Ala Thr Leu Ser Arg Leu Leu Val Arg Tyr
645 650 655

Arg Gly Pro Glu Pro Ser Glu Thr Val Glu Met Phe Glu Gly Glu Lys
660 665 670

Phe Phe Ala Ala Phe Glu Arg Gly Ile Asp Val Asp Ala Asp Arg Pro
675 680 685

Asp Cys Val Asp Gly Arg Ile Cys Phe Val Phe Tyr Ser His Leu Lys
690 695 700

Asn Val Lys Glu Val Tyr Ile Thr Thr Ala Leu Asp Arg Glu Ala Gln
705 710 715 720

Asp Val Arg Gly Gln Val Ser Phe Tyr Arg Gly Ser Leu Pro Val Glu
725 730 735

Val Pro Ala Glu Ala Glu Ala Ala Arg Gln Arg Lys Gly Thr Asp Ala
740 745 750

Leu Trp Met Ala Thr Leu Pro Ile Lys Leu Pro Arg Leu Arg Gly Ala
755 760 765

Gln Gly Ser Gly Gln Gly Thr Asp Phe Ser Leu Met Pro Leu Asn Leu
770 775 780

Gly Asp Ala Glu Thr Gly Phe Leu Thr Gln Ser Asn Leu Leu Ser Val
785 790 795 800

Ala Ser Arg Leu Gly Pro Asp Trp Pro Ala Val Ala Leu His Leu Gly
805 810 815

Met Pro Tyr His Lys Leu Gln Arg Ile Arg His Glu Phe Arg Asp Asp
820 825 830

Leu Asp Gly Gln Val Arg His Met Leu Phe Ser Trp Ala Glu Arg Gln
835 840 845

Thr Gly Gln Pro Gly Ala Val Gly His Leu Val Gln Ala Leu Glu Gln
850 855 860

Ser Asp Arg Arg Asp Val Ala Glu Glu Val Arg Ala Ile Leu Glu Leu
865 870 875 880

Gly Arg His Lys Tyr Gln Asp Ser Ile Arg Arg Thr Gly Leu Ala Pro
885 890 895

```
Glu Asp Ser Thr Leu Pro Gly Thr Ser Ala Ser Gln Thr Pro Glu Ser
            900                 905                 910

Ala Gln Ala
        915


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<212> PRT
<213> Homo sapiens

<400> 2
Met Ala Ala Thr Val Glu Gly Pro Glu Leu Glu Ala Ala Ala Ala Ala
  1               5                  10                  15

Gly Asp Ala Ser Glu Asp Ser Asp Ala Gly Ser Arg Ala Leu Pro Phe
            20                  25                  30

Leu Gly Gly Asn Arg Leu Ser Leu Asp Leu Tyr Pro Gly Gly Cys Gln
        35                  40                  45

Gln Leu Leu His Leu Cys Val Gln Gln Pro Leu Gln Leu Leu Gln Val
    50                  55                  60

Glu Phe Leu Arg Leu Ser Thr His Glu Asp Pro Gln Leu Leu Glu Ala
65                  70                  75                  80

Thr Leu Ala Gln Leu Pro Gln Ser Leu Ser Cys Leu Arg Ser Leu Val
                85                  90                  95

Leu Lys Gly Gly Gln Arg Arg Asp Thr Leu Gly Ala Cys Leu Arg Gly
            100                 105                 110

Ala Leu Thr Asn Leu Pro Ala Gly Leu Ser Gly Leu Ala His Leu Ala
        115                 120                 125

His Leu Asp Leu Ser Phe Asn Ser Leu Glu Thr Leu Pro Ala Cys Val
    130                 135                 140

Leu Gln Met Arg Gly Leu Gly Ala Leu Leu Leu Ser His Asn Cys Leu
145                 150                 155                 160

Ser Glu Leu Pro Glu Ala Leu Gly Ala Leu Pro Ala Leu Thr Phe Leu
                165                 170                 175

Thr Val Thr His Asn Arg Leu Gln Thr Leu Pro Pro Ala Leu Gly Ala
            180                 185                 190

Leu Ser Thr Leu Gln Arg Leu Asp Leu Ser Gln Asn Leu Leu Asp Thr
        195                 200                 205

Leu Pro Pro Glu Ile Gly Gly Leu Gly Ser Leu Leu Glu Leu Asn Leu
    210                 215                 220

Ala Ser Asn Arg Leu Gln Ser Leu Pro Ala Ser Leu Ala Gly Leu Arg
225                 230                 235                 240

Ser Leu Arg Leu Leu Val Leu His Ser Asn Leu Leu Ala Ser Val Pro
                245                 250                 255

Ala Asp Leu Ala Arg Leu Pro Leu Leu Thr Arg Leu Asp Leu Arg Asp
            260                 265                 270

Asn Gln Leu Arg Asp Leu Pro Pro Glu Leu Leu Asp Ala Pro Phe Val
        275                 280                 285
```

```
Arg Leu Gln Gly Asn Pro Leu Gly Glu Ala Ser Pro Asp Ala Pro Ser
    290                 295                 300

Ser Pro Val Ala Ala Leu Ile Pro Glu Met Pro Arg Leu Phe Leu Thr
305                 310                 315                 320

Ser Asp Leu Asp Ser Phe Pro Val Thr Pro Arg Gly Cys Ser Val Thr
                325                 330                 335

Leu Ala Cys Gly Val Arg Leu Gln Phe Pro Ala Gly Ala Thr Ala Thr
            340                 345                 350

Pro Ile Thr Ile Arg Tyr Arg Leu Leu Leu Pro Glu Pro Gly Leu Val
        355                 360                 365

Pro Leu Gly Pro His Asp Ala Leu Leu Ser His Val Leu Glu Leu Gln
    370                 375                 380

Pro His Gly Val Ala Phe Gln Gln Asp Val Gly Leu Trp Leu Leu Phe
385                 390                 395                 400

Thr Pro Pro Gln Ala Arg Arg Cys Arg Glu Val Val Val Arg Thr Arg
                405                 410                 415

Asn Asp Asn Ser Trp Gly Asp Leu Glu Thr Tyr Leu Glu Glu Glu Ala
            420                 425                 430

Pro Gln Arg Leu Trp Ala His Cys Gln Val Pro His Phe Ser Trp Phe
        435                 440                 445

Leu Val Val Ser Arg Pro Val Ser Asn Ala Cys Leu Val Pro Pro Glu
    450                 455                 460

Gly Thr Leu Leu Cys Ser Ser Gly His Pro Gly Val Lys Val Ile Phe
465                 470                 475                 480

Pro Pro Gly Ala Thr Glu Glu Pro Arg Arg Val Ser Met Gln Val Val
                485                 490                 495

Arg Met Ala Gly Arg Glu Leu Gln Ala Leu Leu Gly Glu Pro Glu Ala
            500                 505                 510

Ala Val Ser Pro Leu Leu Cys Leu Ser Gln Ser Gly Pro Pro Ser Phe
        515                 520                 525

Leu Gln Pro Val Thr Val Gln Leu Pro Leu Pro Ser Gly Ile Thr Gly
    530                 535                 540

Leu Ser Leu Asp Arg Ser Arg Leu His Leu Leu Tyr Trp Ala Pro Pro
545                 550                 555                 560

Ala Ala Thr Trp Asp Asp Ile Thr Ala Gln Val Val Leu Glu Leu Thr
                565                 570                 575

His Leu Tyr Ala Arg Phe Gln Val Thr His Phe Ser Trp Tyr Trp Leu
            580                 585                 590

Trp Tyr Thr Thr Lys Asn Cys Val Gly Gly Leu Ala Arg Lys Ala Trp
        595                 600                 605

Glu Arg Leu Arg Leu His Arg Val Asn Leu Ile Ala Leu Gln Arg Arg
    610                 615                 620

Arg Asp Pro Glu Gln Val Leu Leu Gln Cys Leu Pro Arg Asn Lys Val
625                 630                 635                 640
```

```
Asp Ala Thr Leu Arg Arg Leu Leu Glu Arg Tyr Arg Gly Pro Glu Pro
                645                 650                 655

Ser Asp Thr Val Glu Met Phe Glu Gly Glu Glu Phe Phe Ala Ala Phe
            660                 665                 670

Glu Arg Gly Ile Asp Val Asp Ala Asp Arg Pro Asp Cys Val Glu Gly
        675                 680                 685

Arg Ile Cys Phe Val Phe Tyr Ser His Leu Lys Asn Val Lys Glu Val
    690                 695                 700

Tyr Val Thr Thr Thr Leu Asp Arg Glu Ala Gln Ala Val Arg Gly Gln
705                 710                 715                 720

Val Ser Phe Tyr Arg Gly Ala Val Pro Val Arg Val Pro Glu Glu Ala
                725                 730                 735

Glu Ala Ala Arg Gln Arg Lys Gly Ala Asp Ala Leu Trp Met Ala Thr
            740                 745                 750

Leu Pro Ile Lys Leu Pro Arg Leu Arg Gly Ser Glu Gly Pro Arg Arg
        755                 760                 765

Gly Ala Gly Leu Ser Leu Ala Pro Leu Asn Leu Gly Asp Ala Glu Thr
    770                 775                 780

Gly Phe Leu Thr Gln Ser Asn Leu Leu Ser Val Ala Gly Arg Leu Gly
785                 790                 795                 800

Leu Asp Trp Pro Ala Val Ala Leu His Leu Gly Val Ser Tyr Arg Glu
                805                 810                 815

Val Gln Arg Ile Arg His Glu Phe Arg Asp Asp Leu Asp Glu Gln Ile
            820                 825                 830

Arg His Met Leu Phe Ser Trp Ala Glu Arg Gln Ala Gly Gln Pro Gly
        835                 840                 845

Ala Val Gly Leu Leu Val Gln Ala Leu Glu Gln Ser Asp Arg Gln Asp
    850                 855                 860

Val Ala Glu Glu Val Arg Ala Val Leu Glu Leu Gly Arg Arg Lys Tyr
865                 870                 875                 880

Gln Asp Ser Ile Arg Arg Met Gly Leu Ala Pro Lys Asp Pro Ala Leu
                885                 890                 895

Pro Gly Ser Ser Ala Pro Gln Pro Pro Glu Pro Ala Gln Ala
            900                 905                 910
```

<210> 3
<211> 15
<212> PRT
<213> Murine

<400> 3

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Met Ala Ala Val Leu Glu Gly Gln Glu Pro Glu Glu Thr Ala Ala
  1               5                  10                  15
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<210> 4
<211> 14
<212> PRT
<213> Homo sapiens

<400> 4
Met Ala Ala Thr Val Glu Gly Pro Glu Leu Glu Ala Ala Ala
1 5 10

<210> 5
<211> 15
<212> PRT
<213> Murine/Homo sapiens

<400> 5
Ala Leu Gln Arg Arg Arg Asp Pro Glu Gln Val Leu Leu Gln Cys
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<210> 6
<211> 15
<212> PRT
<213> Murine

<400> 6
Arg Ile Arg His Glu Phe Arg Asp Asp Leu Asp Gly Gln Val Arg
1 5 10 15

<210> 7
<211> 15
<212> PRT
<213> Homo sapiens

<400> 7
Arg Ile Arg His Glu Phe Arg Asp Asp Leu Asp Glu Gln Ile Arg
1 5 10 15

<210> 8
<211> 81
<212> PRT
<213> Murine

<400> 8
Leu Leu Ser Val Ala Ser Arg Leu Gly Pro Asp Trp Pro Ala Val Ala
1 5 10 15

Leu His Leu Gly Met Pro Tyr His Lys Leu Gln Arg Ile Arg His Glu
20 25 30

Phe Arg Asp Asp Leu Asp Gly Gln Val Arg His Met Leu Phe Ser Trp
35 40 45

Ala Glu Arg Gln Thr Gly Gln Pro Gly Ala Val Gly His Leu Val Gln
50 55 60

Ala Leu Glu Gln Ser Asp Arg Arg Asp Val Ala Glu Glu Val Arg Ala
65 70 75 80

Ile

<210> 9
<211> 81
<212> PRT
<213> Homo sapiens

<400> 9

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Leu Leu Ser Val Ala Gly Arg Leu Gly Leu Asp Trp Pro Ala Val Ala
  1               5                  10                  15

Leu His Leu Gly Val Ser Tyr Arg Glu Val Gln Arg Ile Arg His Glu
             20                  25                  30

Phe Arg Asp Asp Leu Asp Glu Gln Ile Arg His Met Leu Phe Ser Trp
         35                  40                  45

Ala Glu Arg Gln Ala Gly Gln Pro Gly Ala Val Gly Leu Leu Val Gln
     50                  55                  60

Ala Leu Glu Gln Ser Asp Arg Gln Asp Val Ala Glu Glu Val Arg Ala
 65                  70                  75                  80

Val
```

<210> 10
<211> 161
<212> PRT
<213> Murine

<400> 10

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Cys Leu Ala His Leu Asp Leu Ser Phe Asn Arg Leu Glu Thr Leu Pro
  1               5                  10                  15

Thr Cys Val Pro Glu Leu His Gly Leu Asp Ala Leu Leu Leu Ser His
             20                  25                  30

Asn His Leu Ser Glu Leu Pro Glu Ala Leu Gly Ala Leu Pro Ala Leu
         35                  40                  45

Thr Phe Leu Thr Val Thr His Asn Arg Leu Glu Arg Leu Pro Leu Thr
     50                  55                  60

Leu Gly Ser Leu Ser Thr Leu Gln Arg Leu Asp Leu Ser Glu Asn Leu
 65                  70                  75                  80

Leu Asp Thr Ile Pro Ser Glu Ile Gly Asn Leu Arg Ser Leu Ser Glu
                 85                  90                  95

Leu Asn Leu Ala Ser Asn Arg Leu Gln Ser Leu Pro Ala Ser Leu Ala
            100                 105                 110

Gly Leu Arg Ser Leu Arg Leu Leu Val Leu His Ser Asn Leu Leu Thr
        115                 120                 125

Ser Val Pro Thr Gly Leu Val His Leu Pro Leu Ile Thr Arg Leu Asp
    130                 135                 140

Leu Arg Asp Asn Arg Leu Arg Asp Leu Pro Ala Glu Leu Leu Asp Ala
145                 150                 155                 160

Pro
```

<210> 11
<211> 161
<212> PRT
<213> Homo sapiens

<400> 11

```
His Leu Ala His Leu Asp Leu Ser Phe Asn Ser Leu Glu Thr Leu Pro
```

```
  1               5                  10                  15
Ala Cys Val Leu Gln Met Arg Gly Leu Gly Ala Leu Leu Leu Ser His
            20                  25                  30
Asn Cys Leu Ser Glu Leu Pro Glu Ala Leu Gly Ala Leu Pro Ala Leu
        35                  40                  45
Thr Phe Leu Thr Val Thr His Asn Arg Leu Gln Thr Leu Pro Pro Ala
    50                  55                  60
Leu Gly Ala Leu Ser Thr Leu Gln Arg Leu Asp Leu Ser Gln Asn Leu
65                  70                  75                  80
Leu Asp Thr Leu Pro Pro Glu Ile Gly Gly Leu Gly Ser Leu Leu Glu
                85                  90                  95
Leu Asn Leu Ala Ser Asn Arg Leu Gln Ser Leu Pro Ala Ser Leu Ala
            100                 105                 110
Gly Leu Arg Ser Leu Arg Leu Leu Val Leu His Ser Asn Leu Leu Ala
        115                 120                 125
Ser Val Pro Ala Asp Leu Ala Arg Leu Pro Leu Leu Thr Arg Leu Asp
    130                 135                 140
Leu Arg Asp Asn Gln Leu Arg Asp Leu Pro Pro Glu Leu Leu Asp Ala
145                 150                 155                 160
Pro
```

<210> 12
<211> 46
<212> PRT
<213> Homo sapiens

<400> 12

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Met Ala Ala Thr Val Glu Gly Pro Glu Leu Glu Ala Ala Ala Ala Ala
  1               5                  10                  15
Gly Asp Ala Ser Glu Asp Ser Asp Ala Gly Ser Arg Ala Leu Pro Phe
            20                  25                  30
Leu Gly Gly Asn Arg Leu Ser Leu Asp Leu Tyr Pro Gly Gly
        35                  40                  45
```

<210> 13
<211> 3938
<212> DNA
<213> Murine

<400> 13

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ttgctccaga ggcgggacca aagcgattcc tcacctaccg agacccccctc tgctttcccc 120
atttgacgga ggtgtgaatt gggaattgtg gctcaagagt gtctactagg gaccgcgata 180
tgggacccta gcacaagaac cccaaagtcc ttttggctcc ccttcgcact gtttactgac 240
cttctcgggc acagtgaacg tctgcagtgg aagaaggttc tgggttctcc ccatgcattt 300
gggtgtgaag atagaaagtg tgtccttatt tccagagccc tgtagggatc agggatcact 360
tgggcagatt ccagaattga ggtggaaacc ctctccttgt ccttgatcta agcagagttc 420
tccaagctat gctgttcaga gtcgccctgc ctggtgcccc tcccaggcag aaggatccag 480
cacagatgca agccgtaggg ctgcagtgac cactccactc acgacccctt gtgcccaggg 540
ttcttgggct ggtggtctca agagctcccg ggtttccaag ctgagttatt gttgctctga 600
cctggagggg gagctggtcc cctacagcct actcctcccc ctgcttctcc agcttctatc 660
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agccttgcct aagcaggctt gcttggcaag ctgggacatg tctggtcccc gaggaccttc 720
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aggatgctgc aacatccacc ttggaggctg tggatgccgg gcctggagca ccttttctcc 840
cggctgggaa ccagttgaac ttggacctac gcccaggggg ctgccatcgg ctacagtacc 900
tgtgttccca gcagccccca cagctgctgc aagtggagtt tttgcggctc agcacccatg 960
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aaatgcccag gctattgctg acctcagatt tggacagctt ccttgtgact ccccatggct 1740
gttctgtgac cctggcctgt ggtgtccgcc tacagtttcc agcaggagcc accaccacgc 1800
ctgtcaccat ccactatcga ctctggttgc ccgagccagg cctggtctct ctgggacctc 1860
atgacttcct gcttagtagt gtcctggagc tgcagcccca tggggtggct ttccagcaag 1920
atgtgagctt atggctgctc tttgtccctc cacgagtccg tcgctgtcgt gaggtagttg 1980
tgaggacacg gagtaacaac acctggaatg acctagagac ccaactggag gaagaggcac 2040
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gcccagtatc caacacttgc cttttgccac cagagggagc acttctctgc tcctcaggtc 2160
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tgcaggtagt gcatatggct ggactagagc tgagaaccct tctggaagaa tcagaggcat 2280
cagtaagccc tctgctgtgc ctttcacaga gcggcccccc cagcttccta caacctgtca 2340
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